

ABSTRACTS OF PAPERS ACCEPTED FOR XXVIIth CONFERENCE OF THE INTERNATIONAL SOCIETY FOR FLUORIDE RESEARCH

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FLUORIDE INGESTION AND HEALTH HAZARDS WITH FOCUS ON ANAEMIA IN PREGNANCY AND LOW BIRTH WEIGHT BABIES: GUIDELINES FOR RECTIFICATION

Abstract number:

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Anaemia in pregnancy and low birth weight babies is one of the major public health problems. Though iron and folic acid tablets are distributed to pregnant mothers through antenatal clinics in rural and urban hospitals, it has, unfortunately, not made a difference. The National Family and Health Survey report indicates that the percentage of anaemic pregnant women ranges from 33 to 72%. The anaemic infants born, range from 55 to 85%. The states where such high percentages of anaemia occur are also known for having high fluoride levels in the drinking water and food, and are endemic for fluorosis. Fluoride ingestion can lead to Irritable Bowel Syndrome (IBS)/ Non Ulcer Dyspepsia. Fluoride toxicity induces erythrocytes to undergo degenerative changes and are eliminated from the blood stream prematurely. Loss of microvilli in the gastrointestinal (GI) mucosa is the reason for the non-absorption of nutrients from the diet. Elimination of erythrocytes (echinocytes) from the blood stream and the non-absorption of nutrients result in low haemoglobin / anaemia. However the withdrawal of fluoride led to a regeneration of the mucosa within a very short time frame of 10 days and led to an increase in the hemoglobin (Hb) content. The objective of our study was to monitor and reduce the fluoride intake in pregnant mothers and this is accomplished by testing the fluoride level in the drinking water and shifting to safe sources. The urinary fluoride levels provided an index of the intake of fluoride from food and other sources, and such sources were also withdrawn. This communication reports the results of a study carried out in an endemic area for fluorosis in New Delhi, India. Pregnant women of 20 weeks gestation or below with a hemoglobin of 5–9 g/dL and free from all other health problems (viz., diabetes, heart disease, hypertension, bleeding during pregnancy, renal disease, other medical problems, etc.) were included in the study group. Hemoglobin was estimated along with the urinary fluoride during every visit by the pregnant women to an antenatal clinic, until delivery. The pregnant mothers with low haemoglobin and high urinary fluoride were allocated into two groups using a computerized random sampling procedure. Group I women received guidance for the elimination of fluoride intake and counselling for improving the essential nutrients through diet. Group II women were controls where all the parameters are identical to the Group I women but they did not receive any guidance and counselling about interventions. Group III women, another control group with a low hemoglobin but where the urinary fluoride was within normal range, also did not receive any guidance or counselling about interventions to practise. A reduction in urinary fluoride levels to normal limits following the withdrawal of fluoride sources and rise in the hemoglobin following the improvement in diet were observed in the Group I subjects. The final outcome in the Group I women when compared to Group II and III women was that in over 90% of babies born, the birth weight ranged from 2.5–3.5 kg which is considered as a normal birth weight of babies. In Group II women over 70% of the babies born were under weight, i.e. below 1.25–2.5 kg. In Group III women 50% of the babies were born with a low birth weight. Pregnant mothers require guidance and counselling for the elimination of the toxic substance fluoride and need advice on how to improve their haemoglobin by consuming a diet rich in dairy products, fruits, and vegetables. Although the urinary fluoride was within normal limits in the Group III women, the poor diet, devoid of essential nutrients, led to 50% the babies being born with a low birth weight. The elimination of fluoride and the improvement in the diet so that it is rich in essential nutrients are the two most important interventions to rectify anaemia in pregnant mothers. In conclusion, the importance of the scientific basis for development of a procedure for achieving the goal of improving the anaemia in pregnant mother and thus reducing the incidence of new born with a low birth weight in endemic fluorosis areas in India has been dealt with and results reported. The guidelines for the rectification of anaemia and low birth weight babies born in endemic fluorosis areas may be an impetus for other nations with endemic fluorosis to embark upon similar programmes for the improvement in maternal and child health.

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Keywords: Anaemia; Fluorosis; Low birth weight babies; Pregnancy.

MACROSCOPIC EPIDEMIOLOGICAL INVESTIGATION OF DRINKING BRICK TEA TYPE FLUOROSIS IN CHINA

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The purpose of this investigation was to clarify the understanding of fluorosis due to drinking brick tea in China by studying the extent of the epidemic, the characteristics of the region, and affected population. Comprehensive information was collected in provinces and autonomous regions where drinking brick tea type fluorosis might be prevalent, including Inner Mongolia, Tibet, Sichuan, Xinjiang, the Xinjiang Production and Construction Group, Qinghai, Gansu, Ningxia, and Yunnan. The information included the number of counties (cities) and townships of the regions where brick tea was drunk, together with data on the population, ethnic constitution, the style of production and living, consumption, and the kinds of brick tea used. It was found that brick tea was consumed in 241 counties and 3246 townships. In 87.46% of the townships, the inhabitants in townships had the habit of drinking brick tea for more than 30 years. The study covered 29,038 administrative villages, 2,601 resident communities, 1,504 elementary schools and 2,873 temples with a total population of 31,052,398 and 122,739 monks. A total of 53 nationalities were involved, amongst whom the Han accounted

for 44.86%, Uighur ethnic for 20.98%, Tibetan for 13.34%, Muslim ethnic for 8.15%, Mongolian ethnic for 4.61%, and Kazak for 3.45%. For the whole population drinking brick tea, the farm population accounted for 64.13%, the pastoral population for 8.79%, the farming-pastoral population for 12.53%, and town population for 14.56%. The average consumption of brick tea per year for all the townships investigated was 39267.4 tons, and fu brick tea, green brick tea and black brick tea were the main kinds. Areas with a high brick tea consumption were distributed mainly in the farming and the farming-pastoral regions. The annual per capita brick tea consumption was 3.77 kg in the farming population, and 2.05 kg in farming-pastoral population. The regions with the habit of drinking brick tea are distributed extensively in western China and in this large population those most affected are the pastoral and farming-pastoral groups. Fluorosis due to drinking brick tea type is a serious public health problem in western China.

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THE PATHO-PHYSIOLOGICAL ROLE OF FLUORIDE IONS FROM FLUORIDATION TO FLUORIFICATION IN MEDICINE: ILLUSTRATING THE 1850 LE MILIEU INTERIEUR THEORY

Abstract number: Fluoride (F) has played a great role in biochemistry since its discovery in 1805 by Guy Lussac. It is present in trace amounts in soft tissues and significant amounts in hard tissues like bone and teeth. There are significant levels found in pathological blood vessels. The sources of F intake including fluoridation and the retention of F in bone and teeth will be illustrated in the presentation. Excessive amounts can be the result of intake of F ions from fluoridated toothpaste, oral rinses, tablets, fluoridated water, and in certain foods, such as tea. In 1850 a French chemist Claude Bernard suggested *Le Milieu Interieur* as a model for explaining a mechanism whereby there exists a consistent equilibrium of chemicals, organic and inorganic ions surrounding cells. The internal environment automatically readjusts itself through a process of feedback to cope with demands on the organism. His study explored the internal environment saturated with a multitude of chemical ions including F, both in both the intra- and extracellular fluid (ICF/ECF). This exchange mechanism depends on both a passive and active system. In the passive system, the saturation of F ion levels determines which way F passes in and out of the cell. The active system is dependent on genetic control, i.e. a genetic mechanism exists to determine the intake and outflow of F ions in cells irrespective of the internal environment. The aim of this presentation is to explore the physiological role of F ions in healthy tissue as mother nature intended, with a continuous and prolonged exposure to F ions. The concentration and threshold of F ion pathology in body tissue is discussed and demonstrated with the *Le Milieu Interieur* theory (1850). Pathological cases of (1) atherosclerosis, (2) osteosclerosis, (3) osteoporosis, (4) dental fluorosis, (5) dental caries and (6) thyrotoxicosis are illustrated to raise discussion on what level of F ions does this beneficial process metamorphose into a pathological one, i.e. fluorification.

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Keywords: Fluoridation; Fluorification in Medicine; History of Medicine; *Milieu Interieur* theory.

DAILY TOTAL INTAKE OF FLUORIDE AND THE PREVALENCE OF SKELETAL FLUOROSIS IN TWO VILLAGES IN CHINA

Abstract number: The relationship between the daily total intake of fluoride and the prevalence of skeletal fluorosis was investigated in two villages in Jiangsu Province, China. In the high-fluoride village of Wamiao, 132 adults (average age 52.36 years; water fluoride 2.18±0.86 mg/L, ranging from 0.85-4.50 mg/L); and in the low-fluoride village of Xinhuai, 35 adults (average age 48.11 years; water fluoride 0.37±0.09 mg/L, ranging from 0.21-0.55 mg/L) were investigated in this study. These study subjects were selected according to the daily total intake of fluoride. The prevalence of skeletal fluorosis in Wamiao village was 31.06%, while there were no cases in Xinhuai village. When the study subjects from two villages were divided into five subgroups according to the daily total intake of fluoride, the daily total intake of fluoride was strongly associated with the prevalence of skeletal fluorosis in the form of a significant positive dose-response relationship (regression equation: $y = 2.624 - 6.855x + 3.424x^2$). The Benchmark Dose Lower Bound (BMDL) of daily total intake of fluoride was 2.50 mg F/person/day calculated according to this dose-response relationship, the Reference Dose (RfD) was 2.50 mg F/person/day. In Wamiao village a significant difference was also found between the daily total intake of fluoride in 41 subjects with X-ray detectable skeletal fluorosis and in 91 subjects without X-ray detectable skeletal fluorosis. Gender related differences in the daily total intake of fluoride, household well water fluoride, and the prevalence of skeletal fluorosis were not found in the subjects in Wamiao village. These findings indicate that the daily total intake of fluoride has a significant positive dose-response relationship with the prevalence of skeletal fluorosis in an endemic fluorosis area associated with high-fluoride drinking water. The RfD in this study was lower than that in the national standard of "Hygienic standard for daily total intake fluoride" (WS/T 87-1996) of China.

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village.

BENEFICIAL ROLE OF *CITRUS LIMON* ON BLOOD AND URINE PHYSIOLOGY OF VILLAGERS RESIDING IN FLUORIDE ENDEMIC AREA OF DAUSA DISTRICT

Abstract number: Dausa district of Rajasthan is a fluorosis endemic area, where Khora-kala village contained 5 9.5 ppm fluoride in drinking water. Fluorosis was identified in the villagers according to the method of Susheela (2001), and they were given *citrus limon* (120 g/day) for 30, 90, and 180 days. Blood and urine samples were collected with the help of trained medical staff from these treated villagers in this fluorosis endemic area (9.5 ppm F⁻) and, as a control, in a fluoride non-endemic area (1 ppm F⁻). Blood and urine samples were analyzed for hematology and biochemical parameters using standard techniques. The results of hematology revealed a significant reduction in total erythrocyte count (RBC), packed cell volume (PCV) and hemoglobin (HB) percentage whereas total leucocytes count (WBC) and mean corpuscular volume (MCV) increased significantly as compared to the control group. The levels of serum fluoride, total bilirubin, albumin and globulin, serum glutamic oxaloacetic transaminase (SGOT) and serum glutamic pyruvate transaminase (SGPT) increased significantly, and the concentrations of ascorbic acid and calcium ions declined following fluoride water exposure. Urine fluoride and calcium ions were also found to be elevated significantly in villagers of Khora-kala. Villagers given *citrus limon* 120 (g/day) for 30, 90, and 180 days revealed significant recovery in total RBC, PCV, MCV, Hb, and total WBC as compared to control group. The elevated levels of serum fluoride, albumin and globulin, bilirubin, SGOT, SGPT and serum urea were also restored almost to control level, however urine fluoride concentration increased significantly following *citrus limon* treatment, revealing removal of fluoride toxicity. *Citrus limon* maintained blood and urine physiology almost to normal range due to its anti-oxidant property. The amelioration of fluoride toxicity was observed to be more in six month treatment of *citrus limon*.

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Keywords: Anti-oxidant amelioration, Blood physiology; Serum fluoride; Urine fluoride.

STUDY ON REPRODUCTIVE ENDOCRINE DISTURBANCES IN FLUORIDE EXPOSED POPULATIONS

Abstract number: The purpose of this research is to study the effect of high fluoride in drinking water on Hypothalamic-Pituitary-Testicular axis of adults. SunYing county, located in Kaifeng city of China was selected as the high fluoride study site (F⁻ in drinking water 3.9 mg/L), and ShenLiLou county was selected as the control site (F⁻ in drinking water 1.0 mg/L). Blood and urine samples were collected from 150 individuals who had lived there for more than 5 years. The urine level of fluoride, and the serum level of gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), testosterone (T), and estradiol (E₂) were measured by the RIA method. The serum level of GnRH was not significantly different between the fluoride exposed population and the control group ($P>0.05$). The serum level of LH in the fluoride exposed men was significantly higher than that of the control group ($P<0.05$), and the serum level of T in the fluoride exposed men was significantly lower compared with the control group ($P<0.05$). The serum level of LH was not significantly different in the fluoride exposed women compared with the control group ($P>0.05$). The serum level of T in the fluoride exposed women was significantly higher compared to the control group ($P<0.05$). The serum level of E₂ was not significantly different between the fluoride exposed population and the control group ($P>0.05$). Fluoride can affect the hormone level at each level of the Hypothalamic-Pituitary-Testicular axis, and induce reproductive endocrine disturbance. The reproductive endocrine disturbing effects for males may be more severe than for females.

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Keywords: Fluorosis; Reproductive endocrine disturbing effects; Hormones; Hypothalamic-pituitary-testicular axis.

FLUORIDE CONTENTS IN IRANIAN BLACK TEA

Abstract number: Tea plant (*Camellia sinensis*) accumulates large amounts of fluoride in mature leaves from soils. The fluoride contained in tea is readily released during infusion, and thus is one of the important dietary fluoride sources. Tea drinking after meal is a traditional practice in Iran. In the present study, fluoride contents were investigated in several of Iranian black tea, tea liquor during infusions and drinking water Sources in the one of cities (Behshar city) in the north of the country. In addition, the DMFT index was determined in the 12 years old students in the same city. To determine the fluoride content, sixty tea samples from ten types of tea were randomly purchased, infused in boiling deionized water for 10 minutes and then analyzed by ion selective electrode method. A totally, 120 water Samples were collected and the water fluoride level was estimated, using SPADNS method. The DMFT index of permanent teeth on 300 students aged 12 years was determined, using its standard methods that recommended by WHO. The results showed that the minimum and maximum fluoride content was 35±6 µg/g and 182±20 µg/g for Sedaghat and Sharzad tea respectively. Results indicated that fluoride contents in the infusions accounted for 67.7 to 91% of the total F contents of the original tea leaf samples. The results showed that mean fluoride concentration of drinking water was 0.25±0.06 ppm. The mean DMFT value was 1.48 ± 0.13. This survey has shown that there is no fluorosis.

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Keywords: Black tea; DMFT; Fluoride intake; Fluoride in tea; Iranian Tea.

FLUORIDE CONTENT OF BOTTLED WATERS AVAILABLE IN IRAN

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The aim of this study was to evaluate the fluoride content of bottled drinking waters commercially available in Iran and to report on the accuracy of the labeling of fluoride concentration. 17 randomly selected commercial brands of bottled water were obtained from different supermarkets in Tehran, Iran. Three bottles of each brand were purchased. Following calibration, four tests were conducted on each bottle using SPADNS method with use of DR/2000s. The average reading for each brand was estimated and also compared with the fluoride content printed on the label. The mean (\pm SD) fluoride content of the bottled water samples was 0.174 mg/L with a range from 0.00 to 0.59 mg/L. 76.5% (n=13) of brands tested mentioned the fluoride concentration on the label, and 70.6% (N=12) had a value under 0.42 mg/L. 100% of the brands had a fluoride concentration under 0.6 mg/L. The use of bottled water may be a significant source of systemic fluoride. This article shows that bottled drinking waters in Iran contain low concentrations of fluoride. The manufacturer's labeling of fluoride concentrations may be inaccurate. When prescribing fluoride supplements, dentists should be aware of the fluoride content of bottled drinking waters used by pediatric patients, especially brands with a concentration higher than 0.3 mg F/L.

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Keywords: Bottled water; Fluoride content of bottled water; Iran.

DYSPEPSIA ASSOCIATED WITH FLUORIDATED WATER

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A 77-year-old woman with a ten year history of weight loss and dyspepsia since 1987, poorly controlled with medication, experienced lasting relief and became pain free after ceasing to use, in 1997, fluoridated water containing sodium silicofluoride to give a fluoride ion, level of 0.8 ppm. A reverse osmosis filter was used to remove the fluoride. She was able to cease using antacid and sucralfate medication, gained 6.4 kg weight, and experienced improved health. She attributed the improvement to the use of filtered water and continued to use it on a long-term basis. At follow-up after 10 years, in 2007, aged 87, she remained well and still used filtered water. She gave the filtered water the credit for her wellbeing. She said that the filtered water had been "like magic" to her and the improvement in her health had been "just a miracle." Gastroscopy on 19 September 1989 showed an 8 mm moderately deep ulcer at the junction of the body and antrum and the histology of a stomach biopsy showed an ulcer base, chronic active gastritis and intestinal metaplasia. No diagnostic abnormality was recognized in the duodenum. No evidence of malignancy or campylobacter pylori was present. A further gastroscopy on 14 November 1989 after two months treatment with sucralfate showed a persistent but much smaller ulcer measuring about 3 mm across. The histology was again of severe chronic active gastritis of the antrum of the stomach and campylobacter were identified. She found that she obtained only temporary relief from treatment with sucralfate and antacids. For many years her activities were restricted by abdominal pain and she existed on plain yoghurt. She could eat only about 4 tablespoonfuls of a meal and could not tolerate foods like vegetables. Her symptoms remitted within about two weeks of using filtered water and she has been able to eat everything since then. She has also noted a marked improvement in arthritis that she previously had in her back, shoulders and a temporomandibular joint. No rechallenge tests were done and it is not possible to be sure that the fluoridated water played an aetiological role in the dyspepsia. However, there is a close temporal relationship between her using filtered water and the commencement of a lasting improvement and the clinical pattern is consistent with work relating fluoride toxicity to dyspepsia. Fluoride reacts with gastric hydrochloric acid to form hydrofluoric acid in the stomach. Hydrofluoric acid has penetrating and corrosive properties possibly leading to inflammation, petechiae, ulceration and other mucosal abnormalities in the stomach and proximal small intestine. Enzyme system inhibition may also contribute. Scanning electron microscope studies have shown a loss of mucus droplets and microvilli, a cracked-clay appearance of the duodenal mucosa, and desquamated epithelium in the gastric mucosa.

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Keywords: Dyspepsia; Fluoridated water.

COMPARISON OF WATER EXTRACTION RATES OF FLUORIDE AND METAL ELEMENTS IN VARIOUS CHINESE BRICK TEAS

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The purposes of this study were to determine the concentration and infusion of F, Al, Ca, Mg, Cu, Zn, Fe and Mn in various brick teas, and to compare the differences in the extraction rates of elements in various types of Chinese brick tea in order to find those that were unsafe because of a high content of F and other metal elements. A total of twenty-five brick tea samples from six provinces, where minority nationalities have been drinking large amount of such brick tea in their daily life, were collected and analyzed. Brick tea water infusions were prepared as follows: 0.5 g brick tea powder was placed into a glass conical flask, 100 ml of boiling deionized water was added and the sample was left for 30 min boiling under cover, then the tea infusions was filtered through filter paper into test tubes for analysis. The content of F and other elements in various types of brick tea and their infusions were detected by ion selective electrode method and ICP-OES respectively. Our study found that the highest level of total F was in Kang brick tea (929 mg/kg), followed by Green brick tea (688 mg/kg), Fu

brick tea (471 mg/kg), Black brick tea (467 mg/kg), Mi brick tea (450 mg/kg), Chitsu ping tea (179 mg/kg), Puerh tea (149 mg/kg), Tuo tea (109 mg/kg); the highest level of total Al content was in Kang brick tea (4748 mg/kg), and the least was in Tuo tea (1189 mg/kg); for average extraction rates of brick tea infusions, the highest was F (80.6%), followed by Zn (47.9%), Mg (45.5%), Mn (34.3%), Al (22.8%), Cu (22.6%), Ca (10.3%), Fe (1.9%), the highest of F extraction rate was in Kang brick tea (89.7%), followed by Black brick tea (83.9%), Tuo tea (83.8%), Fu brick tea (82.2%), Chitsu ping tea (79.9%), Mi brick tea (79.3%), Green brick tea (76.6%), Puerh tea (54.8%); the highest of Al extraction rate was in Kang brick tea (27.6%), and the lowest was in Puerh tea (7.6%). The intake of F and other elements from brick tea depends on the type of tea, since the concentration of metal elements and water extraction varies from one type to another. Kang brick tea was found to be the most unsafe tea for tea drinkers.

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Key words: Aluminum; Brick tea; Elements; Extraction rate.

ESTIMATION OF FLUORIDE INTAKE SOURCES AND FLUOROSIS AMONG THE POPULATION LIVING IN THE CHIANG MAI BASIN, THAILAND

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The study area, Lamphun province, is located in the Chiang Mai Basin, Northern Thailand. Due to high fluoride concentrations in the groundwater, the local people have long suffered from dental and skeletal fluorosis. In 2005, we took 133 groundwater samples in the area and found that the groundwater contained 0.0–12.4 mg F/L with an average of 1.8 mg F/L.

Of 129 junior high school students, 38, 28, 24, 10, 16, and 13 students fell into the Dean Index (DI) levels of 0 to 5, respectively. The drinking water contained 0.0–14.8 mg F/L, and the urine 0.0–11.6 mg F/L. There was a good correlation between the DI values and the fluoride concentrations in urine/drinking water. However, for the DI level 1 (questionable), there was a wide distribution of fluoride concentrations in both urine and drinking water samples. A significant number of students had higher fluoride concentrations in their urine samples than in their drinking water. This led to the hypothesis that drinking water is not the only source of fluoride intake. In 2006, an in-depth survey was conducted on 28 junior high school students and their 28 parents/grandparents to determine the main intake pathways and the intake-excretion balance of fluoride among the population in the study area. Food and beverages were collected by the double-portion method for 3 days, and drinking water and cooking water, as well as 24-hour urine, were collected. A majority of the households were using bottled water with low fluoride concentrations, but only a small number of the households were using bottled water for cooking; that is, a majority of households were still using fluoride-rich water for cooking. The maximum fluoride concentration in cooking water was 15.3 mg F/L. In order to estimate the fluoride intake from rice, laboratory experiments were conducted. The rice popularly eaten by the Northern Thai people was soaked in water containing different concentrations of fluoride. The results revealed that the rice can uptake a large portion of the fluoride in water. Hence, rice soaked in fluoride-rich groundwater could be a major source of fluoride intake among the population in the Chiang Mai Basin.

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Keywords: Chiang Mai Basin; Cooking water; Dental fluorosis; Groundwater; Urine.

DENTAL CARIES AND FLUOROSIS AMONG 12-YEAR-OLD CHILDREN WITH DIFFERENT FLUORIDE EXPOSURE IN HEILONGJIANG PROVINCE, PR CHINA

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Heilongjiang Province is one of the drinking water-type endemic fluorosis areas in China. The aim of this study was to evaluate the effect of fluoride on dental caries and dental fluorosis among 12-year-old children exposed to different concentrations of fluoride in the drinking water. 792 study subjects were selected by multi-stage, stratified and random sampling from 6 different areas (three urban and three rural areas). Clinical examination for

dental caries and dental fluorosis was based on WHO methodology. SPSS12.0 software was used for the data analysis. The fluoride concentrations of drinking water in these 6 areas were 0.29 mg/L, 0.40 mg/L, 0.68 mg/L, 0.77 mg/L, 0.80 mg/L, and 1.14 mg/L, respectively. The corresponding prevalence of caries ranged from 28.0 to 44.7% while the community dental fluorosis index (CFI) ranged from 0 to 0.76 in the different fluoride exposure groups. In the study subject group consuming drinking water containing 0.8 mg/L, the caries prevalence was lowest, and significantly different from other areas ($p < 0.05$). The association between fluoride levels in drinking water and caries prevalence showed a negative correlation with a linear regression coefficient of 0.814 (except for drinking water having fluoride level of 1.14 mg/L). It also had a positive correlation with the community dental fluorosis index with coefficient of 0.808. In the area where fluoride level was 1.14 mg/L, the caries prevalence was significantly higher than those areas where fluoride level was 0.29 (the lowest level in all of 6 areas). This study suggested that caries prevalence could be lowered by having adequate fluoride concentration in the drinking water. However, dental caries prevalence could remain high when drinking water containing a high fluoride level as was indicated in this study.

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Keywords: Dental caries; Index of fluorosis; Heilongjiang.

THE LEVEL AND DISTRIBUTION OF FLUORIDE IN THE WATER RESOURCES OF FOUR STATES OF INDIA AND ITS REMOVAL BY NANOFILTRATION

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In four Indian states, Rajasthan, Uttar Pradesh, Maharashtra and Andhra Pradesh, widely varying amounts of fluorides, ranging from 0.1 to 44 ppm, are present in the water resources. Surface water normally has a low concentration of the ion, mostly below 1.0 ppm. The toxic ion is present in excessive concentration in several ground waters in different states of India, especially in Rajasthan and Andhra Pradesh. Consequently millions of people are crippled by the disease of fluorosis. Investigation of the chemical composition of fluoride bearing waters revealed that waters with excessive fluorides usually have high alkalinity and a low calcium content. Laboratory investigations on the dissolution of fluoride bearing minerals tend to show that various geochemical factors contribute to excessive incidence of fluorides in the ground water of these four Indian states. No relationship was noticed between fluorides, pH, conductivity, chlorides, sulphates and nitrates. Analysis of rocks and soils in different localities did not show any correlation with variations in the fluoride concentrations observed. The results of the investigations showed that excessive alkalinity may be responsible for the higher incidence of fluoride in the waters of these four states of India. Laboratory studies on the solubility of fluorite (CaF_2) showed that the solubility decreased with an increasing concentration of calcium. The fluoride problem is associated with ground water since surface waters do not carry significant levels of fluorides. The highest concentration in some wells of Rajasthan in Nagaur district was 44 ppm of fluoride. In Andhra Pradesh the maximum was 20 ppm. In Uttar Pradesh it varied from 0.2 to 1.8 ppm, while in Maharashtra the range was 0.2 to 1.5 ppm. For the mitigation of fluoride levels, the Nalgonda techniques, fluoride uptake methods, and nanofiltration techniques are the most suitable. More research work is required on nanofiltration for the removal of fluorides.

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Keywords: Fluorosis; Ground water; High alkalinity; Nanofiltration membrane.

EFFECTS OF FLUORIDE AND RELATED FACTORS ON THE NERVOUS SYSTEM

Abstract number:

14

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Classical researches of fluorosis are mainly about teeth and bones, however, recent reports also point to an inverse relationship in China between early fluoride ingestion and intelligence, indicating a lower intelligence quotient (IQ) of 8 to 10 points in children living in villages having elevated fluoride intake from food or drinking water. Therefore, the neurotoxicity of fluoride deserves to be studied. The coexistence of other toxic elements and/or a deficiency of essential elements in high fluoride areas should be taken into consideration as well as interactions between elements which might interfere and enhance the incidence of fluorosis. It is well known that arsenic, lead, aluminum and other elements reduce the IQ. These elements will interact with fluoride in a complicated way in humans. In this research, the object was to study the effects of fluoride and other related factors on the nervous system. Thirty-two Wistar rats were divided randomly into four groups (female: male = 3:1). With one group as control, the other three groups were fed with drinking water containing high fluoride only (HiF) (100 mg NaF/L), low iodine only (LI) (0.0855 mg/kg chow); as well as high fluoride (100 mg NaF/L) and low iodine (0.0855 mg/kg) (HiF+LI) to assess the effects on the learning and memory, oxidative stress, DNA damage, and apoptosis in the brain cell of these rats' offspring. After the animal model was established, the rats were allowed to breed, and 36 rats' offspring in each group (female: male = 1:1) were randomly selected. These young rats were fed with drinking water having the similar concentrations of fluoride and iodine as their parents. Compared with the control group, the learning and memory ability of the rats' offspring was depressed by high fluoride, low iodine, or the combination of high fluoride and low iodine. The activity of cholinesterase (ChE) in the brain was affected to some extent in the high fluoride only group and in the low iodine only group, but was significantly affected in the high fluoride and low iodine group. Brain protein in the rats was found decreased in the low iodine only group, and even more in the combined high fluoride and low iodine group. Although Superoxide dismutase (SOD) activity and the malondialdehyde (MDA) contents in the brain of HiF+LI group were significantly higher, the SOD/MDA ratio in this group was lower. DNA damage in the brain cells assessed by the tailing ratio percent was $24.68 \pm 20.81\%$ in the control group, while in the HiF, LI, and HiF+LI groups, it was $90.93 \pm 9.17\%$, $89.04 \pm 4.99\%$, and $92.48 \pm 4.04\%$, respectively. The percent of brain cell apoptosis in the rats' offspring of the three treated groups was obviously higher than that of the control group. These results suggest that learning-memory ability and brain function in rats are affected by high fluoride and low iodine. Oxidative stress in the brain may be one of the causes of this damages. We also studied the effects of co-exposure with fluoride plus arsenic and fluoride plus lead. The results revealed that DNA damage in brain and lower learning ability were more severe in rats treated with high arsenic and high fluoride. It was found that fluoride lowered the learning and memory ability of rats at weeks 6, 8, 10 and 12, while the lead toxicity reflected only on young rats at 6 and 8 weeks of age. These results indicate that fluoride can adversely affect the nervous system, and that the influence of other environmental factors on fluoride should not be ignored.

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Keywords: Arsenic; Iodine; Lead; Nervous system.

POTENTIALS FOR DEVELOPMENTAL FLUORIDE NEUROTOXICITY

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15

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The foetus and the child are particularly vulnerable to pollution. The foetus shares the mother's exposure and accumulated body burden of pollutants, and some chemicals are transferred to the infant via human milk. Occurrence of severe dental fluorosis in a child, whose mother had worked at the Danish cryolite factory suggests that fluoride transfer from mother to child takes place. The central nervous system may be a target organ, as suggested by laboratory animal studies. During early life, cell differentiation, multiplication and migration must happen in a particular sequence and at certain times to create optimal brain functions of the mature organism. Thus, developmental exposure to neurotoxic substances can cause serious disease and also widespread loss of IQ. While fluoride exposure may cause neurotoxicity in adults, the evidence on developmental neurotoxicity in humans is uncertain and is mainly based on studies carried out in China. Exposures were generally assessed on a community basis, and cross-sectional examinations of neuropsychological test performance were related to water-fluoride concentrations. In humans, only five substances have so far been documented as developmental neurotoxicants: lead, methylmercury, polychlorinated biphenyls, arsenic, and toluene. From this evidence, including our own studies on some of these substances, parallels may be drawn that fluoride could well belong to the same class of toxicants, but uncertainties remain. At least 200 industrial chemicals are known to cause brain toxicity in humans, mainly in adults, and they must also be suspected to harm the developing brain. Because of the individual and societal importance of optimal brain function, recognition of developmental neurotoxicity is a public-health priority, and further evidence on fluoride is needed.

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Keywords: Children; Environmental pollution; Neuropsychological tests; Prenatal exposure delayed effects.

OBSERVATION ON FLUORIDE CONTAMINATION IN GROUNDWATER OF DISTRICT BHLWARA, RAJASTHAN AND A PROPOSAL FOR A LOW COST DEFLUORIDATION TECHNIQUE

Abstract number:

16

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India is among the 23 nations around the globe where health problems occur due to excess ingestion of fluoride (> 1.5 mg/L) by drinking water. In Rajasthan, 18 out of 32 districts are fluorotic and 11 million of the population are at risk. An exploratory qualitative survey was conducted to describe the perceptions of the community regarding fluoride and related health problems in central Rajasthan. A study on distribution of fluoride contamination of groundwater and the associated health hazards was performed in 1030 villages of the Bhilwara district of Central Rajasthan. Water samples, totalling 1286, were collected and analyzed for fluoride concentration. The fluoride concentration in these villages varied from 0.2–13.0 mg/L. 756 (73.4%) villages had fluoride concentrations above 1.0 mg/L. 60 (5.83%) villages had fluoride concentration above 5.0 mg/L with maximum number (24 villages, 19.5%) from Shahpura Tehsil. A detailed fluorosis study was carried out in 41 of the 60 villages having fluoride above 5.0 mg/L. Age, sex, and occupation data were also collected. 4252 individuals, above 5 years age, were examined for the evidence of dental fluorosis while 1998 individuals, above 21 years, were examined for the evidence of skeletal fluorosis. The overall prevalence of dental and skeletal fluorosis was found 3270/4252 (76.9%) and 949/1998 (47.5%) respectively. A mild grade of Dean's classification was present in 1016 individuals (23.9%). 374 (8.8%) individuals had a severe grade of dental fluorosis. The Dean's Community Fluorosis Index for the study area in total was 1.62. The maximum CFI, 3.0, was recorded from Surajpura of Banera Tehsil. Individuals with Grade I type of skeletal fluorosis totalled 566 (28.3%) while only 12 (0.6%) individuals had Grade III skeletal fluorosis. In coping with the problem of fluoride-contaminated water, various methods were introduced for defluoridation. However, due to the cost factor or complexity, these methods could not get momentum and there was a need for an alternative low cost method of defluoridation. The author used marble slurry for defluoridation. The method is based on the fact that calcium carbonate, magnesium carbonate and zeolite have a compact crystalline structure with large calcium and magnesium ions surrounded by small oxygen and carbon atoms. The packing efficiency of such crystals is less with large empty spaces. Similarly zeolite has a sieve structure and is a well-known ion exchanger. The fluoride ion is small in size and is a hard base. Calcium ion and magnesium ions are hard acids, which explains the high stability of calcium and magnesium fluoride. These minerals can easily exchange fluoride. Fluoride can also be accumulated as interstitial ion in these crystalline compounds. In Rajasthan there are huge deposits of marble slurry. It is cheap and found to be a good remover of fluoride from drinking water. The minerals may trap fluoride ions easily inside their crystalline structure. As it is well known, the "Pitcher filtration technique" has been used by human beings for the purification of water for a long time. This principle can be successfully used with marble slurry for the effective removal of fluoride. The results showed it was a cheaper and effective method for fluoride removal. The fluoride concentration of 5 mg/L was reduced to 2.1 mg/L by the use of the marble slurry. In an experiment, the fluoride concentration was reduced to 1.85 mg/L from an initial concentration of 3.9 mg/L. This reduction comes near to the tolerable concentration of fluoride in water. The effect of variables, such as contact time, pH, initial fluoride concentration, temperature, and activated adsorbent have been investigated. Fluoride removal was found to be a function of pH, contact time, initial fluoride concentration, dose of adsorbent, bicarbonate, and activated adsorbent. The various empirical relationships have been established for the adsorption process.

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Keywords: Community Fluorosis Index; Defluoridation; Dental fluorosis; Fluoride contamination; Marble slurry; Rajasthan; Skeletal fluorosis.

RISK OF JOWAR CONSUMPTION ASSOCIATED WITH SEVERITY OF DENTAL FLUOROSIS IN NORTH KARNATAKA

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17

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Dental fluorosis is a major public health problem in 18 states of India and the northern parts of Karnataka. Water is the major source of fluoride in these endemic states although food materials also contribute considerable amount of fluoride intake. Many parts of north Karnataka lie in endemic fluoride belts and have high prevalence and severity of dental fluorosis compared to other endemic areas with a similar fluoride concentration in drinking water. Earlier animal experimental studies have reported that Jowar (a type of millet) consumption interacts with fluoride in the body and enhance dental fluorosis. However, studies on the association between Jowar consumption and dental fluorosis are limited in India. This study was carried out to determine the association, (if any) between Jowar consumption and the severity of dental fluorosis. A community based case control study among school children in the age group 12–15 years in the Davangere district in northern Karnataka was carried out in areas with a high fluoride (1.71 ppm) concentration in the drinking water. Sixty-five children (male 54.7%) with severe grades of dental fluorosis [5–9 scores as classified by Thylstrup and Fejerskov Index 1988 (T & F Index)] were selected as cases through the community screening program. 71 children (male 53.5%) with mild grades of dental fluorosis (1–4 scores of T & F Index) and another 60 children (male 41.7%) with no dental fluorosis were selected randomly from the same area from where the cases were selected and included as the two sets of controls. The exposure ascertainment including details of Jowar consumption was done by 24 hour diet recall and the Food Frequency Questionnaire method. Data on other risk factors such as nutrition status, toothpaste usage, tea consumption and other dietary habits were also collected. Multiple regression analysis was done using the SPSS 11 version. Initial results showed that Jowar consumption was significantly associated with a greater severity of dental fluorosis [Adjusted OR 3.00 (95% CI: 1.51 6.09)] in this high fluoride area. There was a significant decrease in urinary fluoride excretion by Jowar consumers compared to non-Jowar consumers. Hence Jowar consumption is associated with a greater severity of dental fluorosis, additional to that due to the presence of fluoride in drinking water.

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Keywords: Dental fluorosis; Jowar; Molybdenum.

THE ANTICARIES EFFECT OF FLUORIDE MOUTH RINSES EVALUATED WITH ANNUAL DMFT INDEX IN CHILDREN AT 12 YEARS OF AGE

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18

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Since 1970s, a school-based fluoride mouth rinses have been used in Niigata prefecture in Japan because fluoride mouth rinses were thought as the second best anticaries procedure to water fluoridation. The reduction in the rate of dental caries has been reported to be 40–80%. Since 2003, this procedure has been used nationwide because of the guidelines by the Ministry of Labor Health and Welfare. The rate of compliance of the procedure was 34% among school children at 4 to 14 years of age in Niigata prefecture and 3.4% among those nationwide in 2006. The officially reported DMFT index of 12-year-old children decreased from 4.61 (1984) to 1.64 (2001) in Niigata prefecture and from 4.68 (1989) to 1.71 (2006) nationwide, during 18 years. Since the decrease in DMFT index in Niigata prefecture was 5 years ahead of the nationwide decrease, a regression line ($Y=0.94X+0.28$) was available by the single regression analysis, in combination with data with a time lag of 5 years. The mean DMFT in Japan were plotted on X axis and those in Niigata prefecture on Y axis, with $R^2=0.97$ and $r=0.99$, $t=21.67$, $p=0.00000$. The rate of decrease in DMFT in Niigata prefecture was almost the same as that in the mean DMFT index in Japan. If fluoride mouth rinses have benefits against dental caries, the rate of decrease in DMFT index must be different between Niigata prefecture and in Japan. Therefore, unknown factors including health education other than fluoride are likely to be involved in the decrease in DMFT index in both cohorts. According to the Cochrane Review (2003), combinations of topical fluoride (toothpastes and mouth rinses) provided a 7% additional increase in the rate of dental caries prevention by mouth rinses, that is not significant statistically significant ($p=0.06$) by meta analysis. The above results suggest that fluoride mouth rinses provide very little or almost no anticaries effect.

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Keywords: DMFT index; Fluoride mouth rinses; Single regression analysis.

RESEARCH OF FLUORIDE IN CHINA: ELIMINATE THE HARMFUL IMPACTS, IMPROVE THE BENEFICIAL EFFECTS

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19

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Fluoride has played a key role in dental caries prevention and osteoporosis treatment for the past 50 years, therefore, fluoridation of drinking water was regarded as one of the great public health achievements in the 20th century. Since excessive ingestion of fluoride through toothpaste or medicine may lead to the onset of fluorosis, contradictions arose throughout the world among the researchers concerning the effects of fluoridation of drinking water. The situation in China is complicated. On one hand, fluorosis induced by the excessive intake of

fluoride through drinking water or air (burning coal rich in fluoride) has been found in many villages of rural areas. On the other hand, due to the high prevalence of dental caries of children in the cities of China, fluoride has also been used to prevent dental caries. Preventive effects have been confirmed by the field test of water fluoridation carried out in Guangzhou, China during 1970s and 1980s. Since the situation of fluorosis in China is severe, the most important work about which researchers are concerned is defluoridation in the epidemic areas of fluorosis in rural areas. As far as the fluoridation is concerned, toothpaste or water with fluoride has been used in some cities of China, although differing views on the merits of this exist among researchers. Suggestions concerning fluoridation provided by most of the researchers of China are also summarized in this article. 1) An appropriate level of fluoride in toothpaste or drinking water should be researched very carefully before fluoridation is widely used. 2) Follow up observations must be carried out in the population which has received fluoridation. The objective is to eliminate the harmful impacts and improve the beneficial effects of fluoride simultaneously.

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Keywords: Defluoridation; Fluoridation; Fluorosis.

REGULATION OF SODIUM FLUORIDE ON THE EXPRESSION OF OPG AND RANKL mRNA IN MOUSE OSTEOBLASTS

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20

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The receptor activator of nuclear factor kappa B ligand (RANKL) and its decoy receptor, osteoprotegerin (OPG), are playing an important role in bone metabolism, which directly control osteoclastogenesis. Our study investigated the expression change of OPG and RANKL mRNA using an *in vitro* osteoblast culture assay. The osteoblasts were derived from mouse calvarial bone and isolated by complete trypsinization and collagenase digestion and cultured in DMEM supplemented with 15% FBS at 37°C containing 5% CO₂. The subcultured osteoblasts were identified by Giemsa and Alkaline phosphatase staining, the mineralized nodule by Alizarin red staining. The different concentrations of sodium fluoride (0, 1.0×10⁻¹², 1.0×10⁻¹¹, 1.0×10⁻¹⁰, 1.0×10⁻⁹ and 1.0×10⁻⁸ mol/L) were exposed to the osteoblasts for 40 h at 37°C containing 5% CO₂, then the total RNA was extracted and reverse transcribed into first-strand cDNA. The cDNA was amplified using OPG, RANKL and β-actin primers by real-time fluorescent quantitative PCR (FQ-PCR) in DNA Engine OpticonTM2 continuous fluorescence detection system. The results indicated that OPG and RANKL were expressed by osteoblasts. However, OPG mRNA expression of mouse osteoblasts in sodium fluoride treated groups were up-regulated against that of control, and the RANKL mRNA expression also increased excepting the group of 10⁻¹² mol/L sodium fluoride. When exposed sodium fluoride of 1.0×10⁻¹² to 1.0×10⁻⁸ mol/L, the relative expression level of OPG/RANKL mRNA were also up-regulated against that of control. Particularly the ratio of OPG/RANKL increased steadily from 1.0×10⁻¹² to 1.0×10⁻¹⁰ mol/L sodium fluoride. Thereafter, it was decreased gradually from 1.0×10⁻¹⁰ to 1.0×10⁻⁸ mol/L sodium fluoride. In conclusion, these *in vitro* data demonstrated that sodium fluoride could regulate the mouse calvarial osteoblasts through the OPG/RANKL system, and also could decrease osteoclastogenesis. This project was supported by National Natural Scientific Foundation of China (NSFC) (No. 30371065 and No. 30571362).

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Keywords: Mice; Osteoblasts; OPG; RANKL, Sodium fluoride.

ROLE OF OXIDATIVE STRESS IN FLUORIDE-EXPOSED OSTEOBLASTS

Abstract number:

21

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We have recently demonstrated that low fluoride stimulated osteoblasts up-regulating proteins that were closely associated with proliferation and oxidative folding, the latter is coupled with a redox reaction, thus there probably existed a closely relationship between cell proliferation and excessive reactive oxygen species (ROS). The present study was designed to investigate whether oxidative stress would attribute to increase fluoride action on osteoblasts viability *in vitro*. Primary calvarial osteoblasts from neonatal Kunming mice were cultured and sub-cultured to the third generation. Osteoblasts were divided into 7 fluoride-treated groups (n=8) and 1 control group (n=8) except for semi-quantitative RT-PCR experiment. Osteoblasts were treated with α-MEM (5% calf serum) containing 0.5, 1.0, 2.0, 4.0, 8.0, 12.0 and 20.0 mg/L of fluoride, respectively. MTT analysis demonstrated a significant increase in cell viability after osteoblasts exposed to low concentration of fluoride (0.52 mg mgF⁻¹/L) for 2472h, and our RT-PCR analysis further indicated up-regulated expression of anti-apoptosis gene bcl-2 and down-regulated expression of pro-apoptosis gene bax; whereas cell viability in the high concentration of fluoride (20 mgF⁻¹/L) was the lowest. An analytical program was performed to assess oxidative stress parameters by biochemical and RT-PCR methods, including the estimation of lipid peroxidation (Malondialdehyde, MDA), and antioxidant enzyme activities [Catalase (CAT), Glutathione peroxidase (GPX), Superoxide dismutase (SOD)]. MDA levels exhibited significant increase in the low fluoride-exposure and the highest fluoride-exposure groups by comparison with the respective control. The activity of CAT and GPX was significantly higher in osteoblasts of the low fluoride-exposure groups, but that showed the lower trend in the high fluoride-exposure groups. It suggested that low concentration of fluoride stimulated activity of antioxidant enzymes in varying degrees, and promoted product of lipid peroxidation accumulated in osteoblasts. We demonstrated here that both low and high dose of fluoride caused active state of oxidative stress in osteoblasts; however, changes of osteoblasts viability are different from the low dose to high dose of fluoride groups, which suggesting that oxidative stress may be induced by the active cells viability under low dose of fluoride action, conversely, it does attribute to inhibit cell viability under high dose of fluoride action.

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Keywords: Catalase; Glutathione peroxidase; Osteoblasts; Oxidative stress; Superoxide dismutase.

EVALUATING MITIGATIVE ROLE OF CURCUMIN AND MELATONIN AGAINST ARSENIC AND FLUORIDE INDUCED GENOTOXICITY

Abstract number:

22

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Conference

Arsenic (As) and fluoride (F) are known ground water contaminants, and threaten the health of many in several countries. The present study was carried out to analyse the protective role of curcumin as well as melatonin on micronuclei induced by arsenic and/or fluoride. Peripheral blood lymphocyte culture (PBLC) was performed using blood from normal healthy individuals within the age group of 20–30 years. Three pro-oxidant groups included As (As_2O_3 1.4 μ M) and F (NaF 34 μ M) added alone and in combination. Melatonin (0.2 mM) and curcumin (7.7 μ M), each co-cultured with As and/or F were the antioxidant groups. Ethyl methanesulphonate (EMS 1.93 mM) was the positive control. As and F alone and in combination showed a remarkable increase in the frequency of micronuclei induction, as compared to control. Curcumin and melatonin co-cultured with As and/or F demonstrated a significant reduction in the micronuclei induction frequency, when compared with the respective pro-oxidant groups. The percentage amelioration calculated was above 70% in all the cases. The values of induced micronuclei for melatonin and curcumin were comparable to the control, however EMS showed a marked increase in micronuclei induction in comparison to control values. From this data, it can be concluded that curcumin and melatonin both exhibit a protective role against the genotoxic potential of As and F. The antioxidant properties of both melatonin and curcumin could be the main driving force for their mitigative role.

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Keywords: Arsenic; Curcumin; Melatonin; Micronuclei.

EXPRESSION OF CORE-BINDING FACTOR α 1 AND OSTEOCALCIN IN FLUORIDE-TREATED FIBROBLAST AND OSTEOBLAST

Abstract number:

23

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Fibroblast (FB), the main type of cell exists in the extra-periosteal soft tissue including tendon and the point linking up the ligament, is in the category of inducible osteogenic precursor cell (IOPC) because of its osteogenesis function under the non-physiological conditions. It is possible that fluoride can induce expression of osteogenic phenotype and enhance the potent osteogenesis function of FB. To study the effects and importance of fluoride on FB in the development of extraperiosteal calcification and ossification of skeletal fluorosis, the expression of osteogenic phenotype in FB line (L929) and osteoblasts (OB) exposed to fluoride, including core-binding factor α 1 (CBFA1) and osteocalcin (OCN), were studied. Fibroblasts and osteoblasts were exposed to different concentrations of fluoride (0, 0.0001, 0.001, 0.1, 1.0, 10.0 and 20.0 mg/L F^-). By using RT-PCR and ELISA methods, the levels of mRNA of CBFA1 and OCN were measured at 48h and protein of CBFA1 and OCN were measured at 2, 4, 24, 48 and 72 h. The data have demonstrated: 1) The content of CBFA1 protein increased obviously in groups of 0.0001, 0.001, 1.0, 10.0 and 20.0 mg/L F^- at 48 h (2.35 \pm 0.08, 2.28 \pm 0.09, 2.32 \pm 0.09, 2.25 \pm 0.08 and 2.28 \pm 0.09) and group of 10 mg/L F^- at 72 h (2.48 \pm 0.22) than their controls (2.13 \pm 0.07 and 2.21 \pm 0.00 respectively). The level of CBFA1 mRNA was much higher in the group of 10.0 mg F^- /L at 48 h (1.29 \pm 0.30) than it was in the control group (1.02 \pm 0.12). 2) The content of OCN protein were increased in fluoride groups of 0.0001, 0.1, 1.0, 10.0 and 20.0 mg/L (2.61 \pm 0.22, 2.28 \pm 0.09, 2.52 \pm 0.18, 2.52 \pm 0.18, 2.42 \pm 0.29, 2.42 \pm 0.18) at 2 h and in groups of 0.001 and 0.1 mg/L F^- (2.35 \pm 0.08, 2.35 \pm 0.22) at 4 h than it was in control (2.17 \pm 0.13); A higher expression of OCN mRNA was also found in the groups with 0.1 and 20.0 mg/L F^- (1.23 \pm 0.06, 1.25 \pm 0.07) than in the control (1.18 \pm 0.05). 3) The expression of CBFA1 and OCN in osteoblasts, treated with same experimental condition as the fibroblasts, was also up-regulated by fluoride, with the same tendency as they had in fibroblasts. Our results showed the increased expression of CBFA1 and OCN in fibroblasts exposed to fluoride and suggested that fibroblast's osteogeneous function induced by fluoride possibly plays an important role in the developing of extraperiosteal ossification of skeletal fluorosis.

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Keywords: Core-binding factor α 1; Fibroblasts; Osteoblasts; Osteocalcin.

ALTERATIONS IN CALCIUM HOMEOSTASIS ON FLUORIDE EXPOSURE IN RAT BRAIN SYNAPTOSOMES AND AMELIORATION BY ANTIOXIDANT SUPPLEMENTATION

Abstract number:

24

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Calmodulin, a multifunctional calcium binding protein, is known to have a strategic role in the efficient functioning of nervous system. The developing nervous system is much more sensitive to fluoride than the adult and pathogenic process in the developing brain involves oxidative stress which perturbs neuronal ion homeostasis by promoting membrane lipid peroxidation. In the present study, cognitive deficits noticed in developing animals on exposure to long term fluoride administration (200 ppm) exhibited altered oxidative stress and membrane lipid peroxidation resulting an impairment in the function of membrane transports (Na^+ - K^+ ATPase/ Ca^{2+} ATPase) and energy (ATP) depletion. Further altered level of calcium binding acidic protein calmodulin and another brain specific acidic protein S-100 in different regions of neuronal tissue indicate altered calcium cascade signaling mechanism (Ca^{2+} / CAM dependent) on fluoride exposure, thus causing impairment in the functional integrity of neuronal membrane and disruption in calcium homeostasis. This could be responsible for neuronal excitotoxicity and apoptosis. The development of pharmacological and dietary interventions may normalize the

toxic manifestations of fluoride. In the present experiment, dietary supplementation of antioxidants, vitamin E, selenium and clinoptilolite gave considerable protection to neuronal tissue and might also protect synaptic plasticity by regulating the expression of neuro-trophic factor/s like calmodulin.

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CHANGED CHOLINERGIC RECEPTORS AND CELLULAR MEMBRANE LIPIDS IN RAT BRAINS AND CULTURAL NEURONS EXPOSED TO EXCESSIVE FLUORIDE

Abstract number:

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It has been indicated that excessive exposure to fluoride can result in dysfunction of the central nervous system, and alternations of mental work capacity and the Intelligence Quotient. In order to reveal mechanisms of brain disorders induced by fluoride, it is necessary to determine the levels of cholinergic receptors and lipid composition and cultural neurons exposed to high-fluoride in the rat's brains. The Wistar rats were fed with different doses of fluoride and the cultured cell lines treated with different concentrations of fluoride. The neurotoxicity, e.g., MTT reduction, lipid peroxidation and protein oxidation, was analysed by photometric measurement; sites of receptor binding by specific radio-labeled ligands; cholinergic receptors, especially nicotinic acetylcholine receptors (nAChRs) at protein or mRNA level by Western blotting or RT-PCR; lipid compositions by high-performed liquid chromatograph. The results showed that a reduction of MTT level, an increase of lipid peroxidation levels and oxidation of protein were detected in cultured cells and brain tissues exposed to fluoride in a dose-dependent manner. In addition, reduction in [¹²⁵I]-bungarotoxin values and [³H] epibatidine binding sites were found. An inhibition of nAChR 7, 4, or 3 at protein level was observed, but with no changes of mRNA levels of these corresponding subunits. Exposure to free radical inducer resulted in increased lipid peroxidation and decreased nAChRs. Furthermore, antioxidants attenuated the inhibition of the receptors and modified lipid compositions induced by fluoride. These findings suggest that the deficit of nAChR might result from modified cellular membrane lipid compositions by influencing the insertion and/or turnover of the receptors. Most importantly, these alterations may be due to the high level of oxidative stress induced by the neurotoxicity of excessive amount of fluoride.

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Keywords: Central nervous system; Cholinergic receptors; Fluorosis; Membrane lipids; Oxidative stress.

STRONG TOXICITY, HYPOCALCEMIA AND HYPERKALEMIA, SEVERE HEPATIC DAMAGE AND RENAL DYSFUNCTION IN OCCUPATIONAL ACCIDENT MODEL OF CADMIUM FLUORIDE IN RATS

Abstract number:

26

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Cadmium fluoride (CdF₂) is commonly used as an insulator for super-high-speed mass telecommunications equipment, and there is a considerable risk that industrial workers will inhale CdF₂ particles. However there are no studies to date that address the health consequences of acute CdF₂ exposure. This study aimed to determine the acute lethal dose of CdF₂ and its effects on the serum electrolytes, liver and kidneys. The effective lethal dose was determined and dose-response study was conducted after intravenous administration of CdF₂ in rats. The dose-response study used doses of 1.34, 2.67, 4.01 mg/kg CdF₂ or saline. Sodium fluoride (NaF) was used to investigate the effect of ionized fluoride (F). Blood samples were obtained from the right carotid artery 5 hours after infusion. Urine accumulated for 5 hours after injection was obtained from an indwelling catheter. The 24h LD₅₀ of CdF₂ was determined to be 3.29 mg/kg. The LD_{50 i.v.} 3.29 mg/kg that we measured for CdF₂ suggests that CdF₂ may have the strongest toxicity among all cadmium compounds. Serum potassium significantly increased in the 4.01 mg/kg group. Serum potassium in the NaF treated group slightly increased as compared with the saline group. Serum calcium significantly decreased in the NaF treated group and the 4.01 mg/kg group. The abnormal changes in serum electrolytes that result from CdF₂ exposure are mainly caused by the effect of F. The AST and ALT activities measured from rats in the 2.67 and 4.01 mg/kg group were significantly higher than from rats in the saline group. In the 4.01 mg/kg group, serum glucose significantly decreased to 50% of the glucose level measured in the saline group. Comparison of the saline and NaF groups did not show any remarkable changes in liver enzymes or serum glucose. Urine volume was significantly decreased in the 4.01 mg/kg group. NAG/Cr and urine glucose increased in the 2.67 and 4.01 mg/kg groups. There were no remarkable changes in these parameters in the NaF group. It was suggested that the liver and renal dysfunctions were caused by CdF₂ rather than by F.

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Keywords: Cadmium fluoride; Hepatic and renal dysfunction; Intravenous infusion; Lethal dose; Serum electrolytes;

EFFECT OF SODIUM FLUORIDE AND SULFUR DIOXIDE ON APOPTOSIS AND APOPTOSIS-DEPENDENT PROTEIN EXPRESSION OF SPERMATOGENIC CELLS IN RATS

Abstract number: 27
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Fluoride and sulfur dioxide are two well known environmental pollutants and when they occur together in certain localities they present a serious threat to animal reproduction. In order to investigate the effects of both pollutants on apoptosis and apoptosis-dependent protein expression in spermatogenic cells, ninety-six adult male Wistar rats were divided randomly into four groups of twenty-four rats each group. One was the control group, and the other three were study groups which were administered, respectively, for eight consecutive weeks, 100 mg NaF/L (45 mg F/L) in their drinking water, SO₂ in ambient air (39.3 mg/m³ SO₂, 4hr/day), and both NaF in drinking water and SO₂ in ambient air together. The results showed that every kind of spermatogenic cell showed apoptosis. This was most severe in the spermatocytes, less in spermiogonia, and the least in the spermatids and sperm, using the method of Terminal deoxynucleotidyl Transferase Biotin-dUTP Nick End Labeling (TUNEL). The immunohistochemistry determination indicated that fluoride and SO₂ led to significant changes of protein expression of p53, bcl-2, and bax in the testis of rats. At week 2 and 6, expression of p53, bcl-2, bax were significantly decreased in all treatment groups, and the expression of bax fell again, while the expression of p53, bcl-2 arose at week 4. At week 8, the expression of p53 was increased, bcl-2 decreased and expression of bax just higher in the NaF+SO₂ group. Compared with the control group, the value of p53/bcl-2 and bax/bcl-2 were markedly higher at week 2, 6, 8 especially at the end of the period. Generally, the trend of the changes of the expression of p53, bcl-2 and bax was parallel with that of apoptosis. Furthermore, the effects of NaF+SO₂ on rat reproduction were more severe than the either NaF or SO₂ on their own. These results suggest that apoptosis of spermatogenic cells induced by the co-occurrence of NaF and SO₂ might be one of the causes of reduced sperm quality in male rats.

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Keywords: Apoptosis; Bcl-2; Fluoride and sulfur dioxide; P53; Spermatogenic cells.

THE SERUM ELECTROLYTES DISORDER, ACID-BASE UNBALANCE AND RENAL DYSFUNCTION AFTER ACUTE EXPOSURE OF LOW CONCENTRATIONS OF HYDROFLUORIC ACID IN RATS

Abstract number: 28
XXVIIth ISFR Conference

Hydrofluoric acid (HFA) is commonly used to wash semiconductors as an acid detergent. It is well known that lethal occupational accidents may occur with high concentrations, such as severe dermal burns or inhalation due to 20-70% HFA. However, the acute effects of low concentrations (<1%) of HFA have not been well researched. The toxicity would be also enhanced by the rapid absorption. The purpose of this study is to investigate the lethal toxicity and harmful systemic effects after acute exposure absorption of low concentration of HFA which contains sublethal doses. The acute toxicity was investigated in a 24-h lethal dose study of intravenous infusion in rats. The lethal dose lowest (LDLo) and LD₅₀ were 13.1 and 17.4 mg/kg, respectively. Harmful systemic effects were also studied 1 h after acute sublethal exposure to HFA. Rats were injected with HFA (1mL) [(0.05%) 1.6, (0.1%) 3.2, (0.2%) 6.4 or (0.3%) 9.6 mg/kg], saline, sodium fluoride (NaF) or HCl solution. NaF and HCl solution concentrations corresponded to the F⁻ and H⁺ concentrations of 0.3% HFA. Regarding the dose-response study, blood urea nitrogen (BUN) and creatinine (Cr) were significantly increased in response to HFA concentrations greater than 0.1%. Acute glomerular dysfunction also occurred at HFA concentrations greater than 0.1%. HCO₃⁻ and base excess (BE) were significantly decreased in the 0.2% and 0.3% groups. Ca²⁺ was significantly decreased, and K⁺ was increased in the 0.3% group. In the NaF and HFA groups, the BUN, HCO₃⁻ and BE were significantly increased. Cr was significantly increased in the HFA group only. Ca²⁺ was significantly decreased, and K⁺ was significantly increased in the NaF and HFA groups. F⁻ exposure would disorder serum electrolytes, acid-base balance and renal function. Lethal effects of HFA are promoted by exposure routes such as inhalation that cause rapid absorption into the body. Even if the concentration is low, HFA can cause acute renal dysfunction, electrolyte abnormalities, metabolic acidosis and a poor prognosis.

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Keywords: Electrolyte abnormalities; Glomerular dysfunction; Hydrofluoric acid; Metabolic acidosis; Sublethal dose.

INFLUENCE OF SUBCHRONIC EXPOSURE TO EXCESSIVE FLUORIDE ON LEVEL OF BONE SIALOPROTEIN EXPRESSION IN RAT BONE TISSUE

Abstract number: 29
XXVIIth ISFR Conference

The purpose of this study was to investigate the influence of subchronic exposure to excessive fluoride on the level of bone sialoprotein (BSP) expression in rat bone tissues. Sixty male Wistar rats were randomly and equally divided into four groups as follows: one control group with 0 mg F⁻/L and three fluoride-treated groups with 50, 100, and 150 mg F⁻/L respectively in their drinking water for three months. At the end of each month five rats from every group were sacrificed for serum, urine, and bone samples. The fluoride electrode method was used to determine the fluoride concentrations in the rat serum, urine and bone samples. The immunohistochemistry assay was used to determine the level of BSP expression in rat bone tissues taken from left one third of distal femur. After immunohistochemical staining, sections were analyzed in a random order with a computer-assisted image analysis system. SPSS 12.0 statistical software package was used to analyze the experimental data. The logarithmic transformation of data was analyzed with one-way Analysis of Variance and SNK test. The rats' urine, serum and bone fluoride levels were significantly increased as the rats' drinking water fluoride

concentration increased and with the experimental period prolonged ($P < 0.01$). The results indicated an obvious dose-response and time-response relationship between fluoride concentration in the drinking water and the degree and incidence rate of rat dental fluorosis, and between the fluoride concentration and BSP expression level ($P < 0.05$). There was significantly negative correlation between the grey value and bone fluoride level, but there was significantly positive correlation between the mean optical density and bone fluoride level. Subchronic exposure to excessive fluoride might result in the increased deposition of fluoride in bone tissue and fluoride excretion in the urine, and lead to an enhanced BSP expression level in rat bone tissues. Our experimental results suggest that the enhanced BSP expression level in fluoride-treated rat bone tissues might play an important role in the pathologic mechanism of skeletal fluorosis since BSP can promote osteoblast differentiation, activation and matrix mineralization.

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Keywords: Bone sialoprotein; Fluorosis; Pathologic mechanism.

FLUOROSIS CAUSED BY INDOOR COAL COMBUSTION IN CHINA: DISCOVERY AND PROGRESS

Abstract number:

30

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Endemic fluorosis caused by indoor coal burning for domestic cooking and heating is found only in China. Effective interventions for the prevention and control of this coal-burning type of endemic fluorosis have not yet been developed. In this study, investigations of endemic fluorosis were conducted through analysis of the fluoride level in the home environment. In the endemic fluorosis areas, local coal having high fluoride concentration is mixed with clay for burning in households. In the endemic fluorosis areas, the coal burning stoves used by households do not have chimneys and smoke containing a high fluoride concentration circulates within the kitchen and the house. The fluoride is absorbed into the moist corn and chilies hanging in the kitchen to dry. As the dried corn and chilies are the local staple diet, the consumption by local inhabitants of these high fluoride containing corn and chilies is the major factor causing endemic fluorosis in these areas. The moist high aluminum- and iron-containing local clay strongly absorbs fluoride. The fluoride content in the corn and chilies and the prevalence of fluorosis increase with an increasing percentage of clay in soil and with greater coldness and humidity in the indoor air. Except for bone coal, the fluorine concentration in coal is not the controlling factor causing fluorosis. For the time being, there are no efficient stoves with chimneys to prevent fluorine releasing into indoor air and at the same time meet the need for cooking, warming, and drying foodstuffs. More attention should be paid to the clay used to make briquettes in order to reduce indoor coal-burning type fluorosis. The project was supported by National Basic Research Program of China (2006CB708513), the National Natural Science Foundation of China (NSFC 40133010) and the Bureau of Science and Technology of Guizhou province.

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Keywords: China; Clay; Coal; Fluorosis.

MELATONIN AMELIORATES FLUORIDE INDUCED TOXICITY IN THE LIVER OF MICE

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31

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The present study was designed to investigate the protective effect of melatonin (MLT) against fluoride induced toxicity in liver of adult female albino mice. The animals were divided into four groups. Group I served as control; Group II animals were injected melatonin (10mg/kg body weight; i.p.); Group III animals were orally administered sodium fluoride (NaF) (10mg/kg. body weight); Group IV animals were pretreated intraperitoneally with melatonin and after 30 minutes NaF was administered orally. NaF treatment brought about a significant decrease in the body weight and liver weight. An increase in the level of lipid peroxides along with a concomitant decrease in the levels of total ascorbic acid (TAA), glutathione (GSH) and protein was observed. Further activities of enzymes like succinate dehydrogenase, acid phosphatase and alkaline phosphatase were found to be declined in fluoride administered group of mice. But the levels of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were increased indicating liver toxicity. Pretreatment of melatonin to NaF treated mice did not reveal significant alterations in all the above mentioned indices as compared to control. Histological changes included were signs of necrosis, granulization, and nuclear pyknosis. Disorganisation of hepatic cords was also noted along with cellular degeneration. These changes were correlated with biochemical changes found by NaF treatment. Such changes were not indicated in MLT injected mice, revealing its protective role. Thus the results obtained from the present study suggested a prophylactic action of melatonin against fluoride induced hepatic toxicity.

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Keywords: Amelioration; Liver; Melatonin; Toxicity.

THE EFFECTS OF CALCIUM AND PROTEIN ON FLUORIDE METABOLISM BALANCE OF RATS

Abstract number:

32

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It has been well established that calcium and protein can against the endemic fluorosis. In order to understand the effects of those substances on the fluoride metabolism balance, the animal experiment was carried out. In this study, total 71 Wistar rats were divided into three groups, 21 rats in each group: group I were fed normal food, group II were fed normal food with added 0.5% calcium (CaCO₃), group III were fed normal food with added 0.4% protein (tyrosine), and all groups drank 100 mg/L fluoride water (NaF). One of the rats in each group was put into the metabolism cage for a 24-hrs metabolism balance experiment and, for the next 24 hrs, the rat was replaced with another rat from the same group respectively. The rats both in the rearing cage and metabolism cage ingested the same food and water freely. During the 24 hrs, the amount of drinking water and food drunk and eaten by the rats in the metabolism cages were recorded, and all the urine and feces of these rats were collected. 7 of the rats in each group were sacrificed after 4 weeks (one month), 9 weeks (two month), and 14 weeks (three month) respectively. The blood serum and bone samples were collected. All samples were examined by fluoride selective electrode. The results showed that 1) with feeding calcium-rich and protein-rich food, the rats ingested markedly less water and food than the normal food group at one month and two months; 2) the urinary fluoride content for groups II and III was higher than that for group I, but there was not a statistically significant difference between the groups; 3) the calcium-rich food increased the rate of fluoride exclusion through feces, and protein-rich food accelerated the excretion rate of fluoride from urine; 4) the skeletal fluoride content of group I was markedly higher than that of group II and group III. The calcium-rich and protein-rich food decreased the fluoride accumulation into bone; 5) the serum fluoride content of group I was higher than that of group II at three months, and markedly higher than that of group III at the first and second months, but it was not statistically significant at three months. It was concluded that calcium and protein not only enhance the excretion of fluoride but also reduce the fluoride uptake or storage. Based on the morphologic observations, however, at the level of 100mg/L fluoride in drinking water, the protective effects of calcium and protein against fluoride toxicity are very limited.

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Keywords: Calcium; Fluoride metabolism balance; Protein; Rats.

EFFECTS OF FLUORIDE ON VIABILITY AND CYTOKINE PRODUCTIONS OF HEREDITARY NEPHROTIC MICE AFTER ORAL ADMINISTRATION FOR ONE MONTH

Abstract number:

33

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The ICR-derived glomerulonephritis (ICGN) mouse is a strain of mice with a hereditary nephrotic syndrome and is considered a good model of human idiopathic nephrotic syndrome. Since the kidney filters fluoride from the blood and subsequently excretes it into the urine, the effects of fluoride on mice with nephrotic syndrome are of interest. The viability of mice and the production of cytokines, which are related to bone metabolism were investigated. Female ICGN mice with the nephrotic syndrome were exposed to fluoride at the concentrations of 0, 25, 50, 100 and 150 ppm in their drinking water for 1 month. The development of the nephrotic syndrome in each ICGN mouse was confirmed by the determination of BUN in the serum before the administration. As the controls, female ICR mice, the type of progenitors of ICGN mice, were also exposed to fluoride at the concentrations of 0 and 150 ppm in the drinking water for 1 month. After the exposures, liver, kidney and spleen of each mouse was sampled and weighed. Splenic cells were divided into macrophages, T cells and B cells. The divided cells were activated and incubated. The concentrations of tumor necrosis factor (TNF) and interferon- (IFN-) in the supernatant of the cells were determined by ELISA. All ICGN mice exposed to 150 ppm of fluoride were dead before the end of administration. There were no significant differences in body weight among the groups of ICGN mice or between the groups of ICR mice. The mean value of relative spleen weight of ICGN mice exposed to 100 ppm of fluoride was significantly lower than that of the control. There were no significant differences in any cytokines in the supernatant among the ICGN groups, and neither were there any significant differences in relative organ weight or the cytokine levels between the ICR groups. These results showed that the effects of fluoride were more serious for the mice with a nephrotic syndrome compared to the control and may suggest that renal failure may be an important factor to be checked in human exposure to fluoride.

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Keywords: Cytokines; ICGN mice; *In vivo* effects; Nephrotic syndrome; Spleen.

EFFECT OF NUTRITION ON ALLEVIATION OF FLUOROSIS

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34

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Epidemiological investigations demonstrate that the endemic fluorosis is mainly prevalent in underdeveloped areas, particularly those with malnutrition. Based on these investigations, we studied the effects of fluoride and malnutrition on the skeletal and immunologic systems. Two studies were performed in rabbits and rats, respectively. 80 healthy one-month-old rabbits were divided into four equal groups of twenty in each group

with a 1:1 female/male ratio. Group 1 was fed with a malnutrition control diet (protein 8.58%, calcium 0.49%); Group 2 was given a malnutrition control diet with added fluoride (protein 8.58%, calcium 0.49%, F⁻ 200mg/kg); Group 3 was given a protein-rich malnutrition diet with added fluoride (protein 18.41%, calcium 0.46%, F⁻ 200 mg/kg); and Group 4 was given a calcium-rich malnutrition diet with added fluoride (protein 8.35%, calcium 2.23%, F⁻ 200 mg/kg). Praxiology, organization ultrastructure, serum biochemical composition and vitodynamics parameters were observed. The results indicated that with malnutrition a high fluoride diet inhibits growth in rabbits, damages the skeletal system and impairs immunologic function. Protein or calcium supplementation played a protective role to some degree on fluoride toxicity. 150 Wistar rats were divided into 5 groups, with 30 in each group, with random selection and a 1:1 female/male ratio. Group 1 was fed a normal control diet (protein 19.07%, calcium 0.99%); Group 2 was fed with a malnutrition control diet (protein 11.99%, calcium 0.28%); Group 3 was given a malnutrition control diet, and drunk double-distilled water with added F⁻, 100 mg F⁻/L, (protein 11.99%, calcium 0.28%); Group 4 was given a protein-rich malnutrition diet, drunk double-distilled water with added F⁻, 100 mg F⁻/L, (protein 27.12%, calcium 0.29%); and Group 5 was given a calcium-rich malnutrition diet, drunk double-distilled water with added F⁻, 100 mg F⁻/L, (protein 11.95%, calcium 2.07%). The effects of the malnutrition on fluoride-induced growth, tibia histomorphometry and serum skeletal metabolism-related hormones in the rats were observed. Protein or calcium supplementation can alleviate fluoride toxicity to some degree. In conclusion, fluoride damages skeletal and immunologic function in herbivores and rodents. The ameliorative effects of protein or calcium supplementation in fluorosis animals were obvious throughout the treatment.

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Keywords: Bone; Calcium; Immunologic function; Nutrition.

BENEFICIAL EFFECT OF A MAGNESIUM COMPOUND ON FLUORIDE TOXICITY IN RABBITS

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35

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The present study has been undertaken to assess the possible benefits of the administration of a magnesium (Mg) compound (magnesium hydroxide or Milk of magnesia) to prevent fluoride (F) toxicity in simultaneous treatment with fluoride. To this end, New Zealand white rabbits aged 2 to 3 months were taken and divided into three groups [group 1 (control), group 2 (fluoride fed group) and group 3 (fluoride + magnesium fed group)].

Fluoride 20mg/day/rabbit was fed to group 2 animals and 40mg Mg compound was fed to group 3 along with 20mg F/day /rabbit. Group 1 animals received a control diet and water for 7 months. After 7 months of treatment, 24 hr urine and fecal were collected for three consecutive days. Blood samples were collected at the end of the 7 months for various biochemical and hematological parameters. The results of the study revealed that the serum fluoride was significantly higher ($p < 0.01$) in group 2 than group 3 and 1. However, serum F was significantly higher in group 3 as compared to group 1. The serum total alkaline phosphatase was significantly higher ($p < 0.01$) in group 2 than group 1 and 3. Fecal F was significantly higher ($p < 0.01$) in group 3 as compared to group 1 and 2. However, it was significantly higher in group 2 as compared to group 1. Diet and water intake in group 2 was lower as compared to group 1 and 3. Body weight was significantly reduced in group 2 as compared to group 1 and 3 at the end of the 7th month. Other parameters like urinary and serum Ca, Mg, Zn, Cu and bone specific alkaline phosphatase, osteocalcin and 25 hydroxy Vitamin D were not changed along with hematological parameters in the experimental groups as compared to the control group. In conclusion, the feeding of Mg compound reduces fluoride toxicity by increasing faecal fluoride, which is shown by F having much less effect on the body weight and serum alkaline phosphatase.

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Keywords: Beneficial effect of Mg compound, Fluoride toxicity.

EFFECT OF NUTRITION AND FLUORIDE ON THYROID GLAND IN RATS

Abstract number:

36

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The thyroid gland is one of the most sensitive organs in its histopathological and functional responses to excessive amounts of fluoride. Epidemiological investigations reveal that fluorosis and malnutrition frequently coexist in developing countries, such as China and India. Thus study of thyroid hormone metabolism was undertaken to help understand the role of fluoride toxicity in malnutrition. Two experiments were designed to investigate the

effects of iodine (I), protein (Pr) and calcium (Ca) malnutrition and nutrition supplementation on thyroid function in rats. Thirty-two Wistar rats were divided randomly into four groups of eight having six females and two males in each group. The rats were exposed to high fluoride drinking water (45 mg F⁻/L from 100 mg NaF/L), low dietary I (0.0855 mg/kg), or both together in order to assess the effects on the thyroid gland of the offspring rats. After the animal model was established, the offspring rats were used for the experiment. The treatments for the offspring rats were the same as that of their parents. Thyroid cell DNA damage was determined by means of single cell gel electrophoresis (SCGE) and function parameters were also determined. Results suggested that the DNA damage and thyroid function derangement were induced by high fluoride, low iodine, or both together. Exposure time was one important factor on thyroid function deprivation induced by fluoride. One hundred and forty-four 30-day-old Wistar albino rats were divided randomly into six groups of twenty-four: Groups NC and NC+F were given normal nutrition diet (17.92% Pr, 0.58% Ca); Group LPrLCa and LPrLCa+F were given the malnutrition diet (10.01% Pr, 0.24% Ca), Group HPrLCa+F was given the protein-rich malnutrition diet (25.52% Pr, 0.25% Ca); and Group LPrHCa+F was given the calcium-rich malnutrition diet (10.60% Pr, 1.93% Ca). All these diets were isoenergetic and groups NC+F, LPrLCa+F, HPrLCa+F and LPrHCa+F were given high fluoride (150 mg F⁻/L from 338 mg NaF/L) in drinking water.

Body weight and weight gain of rats were obviously inhibited by excessive fluoride ingestion, Pr or Ca supplementation significantly alleviated the fluoride-induced decrease in the case of malnutrition. Excessive ingestion of fluoride, significantly decreased serum thyroid hormone levels in rats given normal nutrition, whereas, in the case of malnutrition, serum thyroid hormone levels were markedly increased. Interestingly, Pr or Ca supplementation markedly inhibited the increase. The dramatic changes of thyroid hormone level induced by excessive fluoride ingestion between normal nutrition and malnutrition indicates that the effect of Pr, Ca and energy levels on thyroid function in fluorosis rats is not negligible.

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Keywords: Nutrition; Thyroid gland.

FLUORIDE DISTRIBUTION AND MODELING USING BEST SUBSET PROCEDURE IN NAGOUR DISTRICT OF CENTRAL RAJASTHAN, INDIA

Abstract number:

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India is among the 23 nations around the globe where health problems occur due to the presence of high concentrations of fluoride, > 1.5 mg/L, in drinking water. In Rajasthan, 18 out of 32 districts are fluorotic and 11 million of the population are at risk. An exploratory qualitative survey was conducted to describe the community perception of fluoride and related health problems in central Rajasthan. A study on distribution of fluoride contaminate in groundwater was performed in 750 villages of Nagour district of Rajasthan by collecting 871 water samples. Fluoride concentration in the district varied from 0.1–19.0 mg/L. 510 (68%) villages had a fluoride concentration above 1.5 mg/L. 142 villages, (18.93%), were in category I in which the fluoride concentration was below 1.0 mg/L, the maximum desirable limit of standards for drinking water recommended by the Bureau of Indian Standards (BIS) in IS: 10500, 1991. Out of 750 villages of the Nagour district, 98 villages (13.06%) had a fluoride concentration between 1.0–1.5 mg/L and fell in category II. The maximum permissible limit of fluoride in the standard for drinking water is 1.5 mg/L (IS: 10500; 1991). About 44% of the population of 322 villages (42.93%), consume water with a fluoride concentration between 1.5–3.0 mg/L, which is above the maximum permissible limit as recommended by BIS. In 22 villages (2.93%) the fluoride concentration in the groundwater was above 5.0 mg/L. The fluoride concentration in the district Nagour is not uniform due to variations in the presence and accessibility of fluoride bearing minerals to water and the weathering and leaching process. The best subset procedure, based on R^2 and F values, is used in model dissemination for predicting the fluoride concentration. Regression models developed are successful in explaining variation in fluoride concentration using the routinely measured parameters of sodium, alkalinity, and potassium. The predicted values of fluoride were compared with the observed values and a reasonably good matching was obtained. From the statistics arrived at and the results / interpretation, two separate models are required to predict the fluoride concentration for both shallow and deep aquifers. The model consisting of the subset of (Na+alk+K) for deep aquifers is different from the model consisting of a single parameter, alkalinity, for shallow aquifers. The developed model for deep aquifers may be used for prediction of the observed values for fluoride concentration.

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Keywords: Bureau of Indian Standard; Fluoride distribution; Modeling; Rajasthan.

ENDEMIC FLUOROSIS THROUGH DRINKING WATER AND GEOLOGICAL ENVIRONMENT IN THE NORTHEAST OF CHINA

Abstract number:

38

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This article expounds the distribution of endemic fluorosis areas caused by drinking water in the Northeast of China. It is believed that the main factors which contribute to the distribution of the endemic fluorosis areas are geological morphostructure and hydrogeological environment. The authors have discussed the relations between the endemic fluorosis prevalence and factors of geological environment, and proposed the counter measures and ways to prevent fluorosis as well as to provide safe drinking water in these areas.

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Keywords: China, Northeast; Endemic fluorosis; Geological environment.

RELATION OF COL1A2 GENE POLYMORPHISMS TO DENTAL FLUOROSIS IN CHILDREN EXPOSED TO FLUORIDE

Abstract number:

39

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Conference

The purpose of this study was to investigate the relationships between dental fluorosis, polymorphisms in the *COL1A2* gene, and serum calcitropic hormone levels. We conducted a case-control study among children between 8 and 12 years of age with ($n=75$) and without ($n=165$) dental fluorosis in two counties in Henan Province, China. The *PvuII* and *RsaI* polymorphisms in the *COL1A2* gene were genotyped using the PCR-RFLP procedure. Calcitonin and osteocalcin levels in the serum were measured using radioimmunoassays. Children carrying the homozygous P allele of *COL1A2 PvuII* had a significantly increased risk of dental fluorosis (OR=4.85, 95%CI: 1.22–19.32) compared to children carrying the homozygous p allele in an endemic fluorosis area (EFV). However, the risk (OR=1.07, 95%CI: 0.45–2.52) was not elevated when the control study subjects

were selected from a non-endemic fluorosis area (NEFV). In addition, the fluoride levels in urine and the osteocalcin levels in serum were found to be significantly lower in the control group from non-endemic areas compared to those of the study group. However, the differences in fluoride and osteocalcin levels were not observed when compared with a control group from endemic fluorosis areas. This study provides the first evidence of a correlation between polymorphisms in the *COL1A2* gene and dental fluorosis in high fluoride exposed populations. Future studies are needed to confirm this correlation.

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Keywords: Calcitonin; *COL1A2* gene polymorphisms; Dental fluorosis; Osteocalcin; Type I collagen.

EFFECT OF FLUORIDE DOSAGE AND NUTRITION ON RIB COLLAGEN GENE EXPRESSION

Abstract number:

40

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In order to determine the effect of fluoride on collagen protein gene expression in malnutrition area, two studies were performed in an industrial fluoride pollution area, a low fluoride area and under laboratory conditions respectively. Eight healthy female Inner Mongolia cashmere goats were divided randomly into two groups. To explore the effect of industrial fluoride pollution on the expression of the two collagen genes (*COL2A1* and *COL1A2*) in their rib cartilage, the control group of 3 goats was pastured in a safe low-fluoride area for 24 months and the study group of 5 goats was sent to pasture in Baotou, an industrial fluoride pollution area, for 24 months. At the end of 24 months, the *COL2A1* and *COL1A2* gene expression levels in the rib cartilage of the control and study group were quantified by conventional reverse transcription polymerase chain reaction (RT-PCR) and real-time RT-PCR. The results indicated that industrial fluoride pollution can affect the expression of the *COL2A1* and *COL1A2* gene in rib cartilage of Inner Mongolia cashmere goats, inducing high expression levels of the two genes. To study the effect of fluoride on rib collagen gene expression in laboratory, 32 healthy one-month-old New Zealand rabbits were randomly divided into four equal groups of 8 in a 1:1 female/male ratio. With one group as control with a marginally low protein (Pr, 8.58%) and low calcium (Ca, 0.49%) diet, the other three groups were given, respectively, for up to 120 days: (1) high fluoride 200 mg F⁻/kg dry feed plus the same low Pr and low Ca control diet, (2) high F plus high Pr (18.41%) and low Ca (0.46%) in their diet, and (3) high F plus low Pr (8.58%) and high Ca (2.23%). After 60 and 120 days, two male and two female rabbits were randomly selected from each group and sacrificed for the quantification of the *COL1A1* gene expression by the real-time RT-PCR method. The results showed that the rib *COL1A1* gene expression level in the high F group decreased compared to the control, whereas in the high F plus high Pr and the high F plus high Ca groups, both the gene expressions increased in comparison with the high F group plus low Pr and low Ca. In conclusion, the difference in fluoride dosage is a key factor in rib collagen gene expression. Low F level can stimulate collagen gene expression and high F has the opposite effect. Furthermore, adequate Pr and Ca nutrition can provide protection against F toxicity.

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Keywords: Collagen gene expression, Nutrition, Real-time PCR.

EXPRESSION OF OSTEOPROTEGERIN AND OSTEOPROTEGERIN LIGAND IN THE BONE OF FLUOROTIC RATS

Abstract number:

41

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Conference

Two hundred and seventy one-month old SD male rats were divided randomly into six groups at forty-five rats in each group. The rats in the three calcium levels control groups drank deionized water, fed on AIN-93G purified rodent diet containing three calcium levels, low calcium 1000mg/kg, normal calcium 5000mg/kg and high calcium 20000mg/kg respectively, and the rats in study groups drunk high fluoride water (100mg F/L from NaF) having the same levels of calcium mentioned above. The aim was to study the effects of fluoride on bone turnover and the withdrawal or synergistic effects of calcium, especially the expressions of osteoprotegerin (OPG) and osteoprotegerin ligand (OPGL) messenger RNA in the bone of rats for 2 months, 4 months and 6 months. Although the expression levels of OPG and OPGL mRNA in the distal femur metaphysis of fluorotic rats showed no obvious change compared with those of control groups, the OPGLmRNA/OPGmRNA ratios were significantly increased at 6 months. The activities of serum tartrate-resistant acid phosphatase (StrACP) of the fluorotic rats during the experimental periods were significantly increased, the activities of serum calcitonin (CT) of the fluorotic rats at 6 months were significantly decreased, and both calcium deficiency or excess intensified these changes. The activities of serum Gla-containing protein (BGP) and Alkaline phosphatase (ALP) of the fluorotic rats showed no obvious change, but the excess calcium decreased the BGP activity. Our results indicate that OPG and OPGL may play an important role in the bone turnover of fluorotic rats, for the higher ratios of OPGLmRNA/OPGmRNA may enhance osteoclast formation and induce osteoclastic bone destruction. Other results show that bone resorption enhancement is the main change in bone metabolism in fluorosis in rats and that only adequate calcium has a beneficial effect on bone metabolism.

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Keywords: Bone, Fluoride, Calcium, OPG, OPGL.

THE mRNA EXPRESSIONS OF FAS, FASL, CASPASE-8 AND CASPASE-3 IN NaF-INDUCED APOPTOSIS IN SH-SY5Y CELLS

Abstract number: 42
XXVIIth ISFR Conference

In order to investigate the mechanism of fluoride-induced central nervous system damage, human neuroblastoma (SH-SY5Y) cells were incubated with different concentrations of NaF (20, 40, 80 µg/ml) for 24 h *in vitro*, the cell viability and mRNA expressions of Fas, FasL, Caspase-8 and Caspase-3 were measured. The results shown that NaF inhibited cell viability, increased the mRNA expressions of Fas, FasL, Caspase-8 and Caspase-3 at 40 µg/ml and above exposure dose respectively ($P < 0.05$ or $P < 0.01$). The present study indicates that NaF may inhibit cell viability and increase the mRNA expressions of Fas, FasL, Caspase-8, and Caspase-3. Fas, FasL, Caspase-8, and Caspase-3 may play an important role in NaF-induced apoptosis in SH-SY5Y Cell.

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Keywords: Apoptosis; Caspase-3; Caspase-8; Fas.

DISTRIBUTION OF FLUORINE AND ARSENIC IN THE CLAY AND SOIL IN THE FLUOROSIS AREA OF SOUTHWEST CHINA

Abstract number: 43
XXVIIth ISFR Conference

The fluorosis area of southwest China is terrain of karst. To study the distribution of fluorine and arsenic in the clay and soil in southwestern China fluorosis area, more than 600 samples of the bedrock, soil, and clay which were used as additives for coal-burning to dry the corn by local residents were collected from the endemic fluorosis area and non-affected area in the eastern Yunnan Province, Guizhou Province, Chongqing City, southwest China. Some cave clay and soil were sampled from the limestone area in North China Plate. The concentrations of fluoride and arsenic were measured. The results showed that the clay in limestone caves and cracks had a exceedingly high fluoride and arsenic content in both the southwest China karst area and North China. The fluoride content in the clay was generally more than 800 mg/kg with the majority being between 1000–1800 mg/kg. The arsenic content in the clay ranged from 14.07 to 59.76 mg/kg, with the majority of the samples having more than 15 mg/kg. The clay in southwest China had a broad range of fluoride and arsenic content. Fluoride ranged from 367 mg/kg to 2435mg/kg, with the majority of samples being 800–1200 mg/kg and with an average of 1084.2mg/kg. Arsenic ranged from 5.71 to 51.95 mg/kg, with the majority of the samples having more than 15 mg/kg and an average of 20.16 mg/kg. The clay with the highest fluoride and arsenic content was found in limestone caves and cracks in areas of different ages in the limestone bed. They are residues that have been left of the limestone strata, a special outcome of the karst evolution. The fluoride and arsenic content in the farming soil of the karst area of southwest China was the same as that of clay. The fluoride and arsenic content in the farming soil and cave clay had a close relationship to the lithology of the bedrock. It was found that the fluoride and arsenic levels in the cave clay and soil were higher when the limestone was purer. The soil in the distribution area of marl and calcium mudstone generally had a low content of fluoride and arsenic, but in the black shale area, the fluoride and arsenic content varied greatly. This work was supported by the National High-Tech R & D Program (863 Program) (2004AA601080, 2006AA06Z380) and was a Key Project of the National Natural Science Foundation of China (90202017).

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Keywords: Clay; Endemic fluorosis; Fluorine and arsenic; China, Southwestern; Karst area.

FLUORINE AND SULFUR POLLUTION FROM INDOOR COAL-COMBUSTION AND ITS AMELIORATION IN SOUTHWEST CHINA ENDEMIC FLUOROSIS AREAS

Abstract number: 44
XXVIIth ISFR Conference

“Coal-burning fluorosis”, which is caused by indoor coal-combustion, is very serious in southwest China. Yuqing village, in Zhaotong, Yunnan province, one of serious endemic fluorosis areas, was selected as experiment site during October 1–20, 2006, just after the corn was harvested, and all families dried the corn by burning coal mixed with clay. We identified 35 families and divided them into 7 groups: A, B, C, D, E, F, G, according to the fluoride content in different kind of clay which was mixed with the coal: M clay which has more than 1000 ppm fluoride content was mixed with coal in groups A and D, T clay which has 360–700 ppm fluoride content are mixed with coal in groups B and E, and P clay with about 800 ppm fluoride level was mixed with coal in groups C and F. At the same time, a special Ordovician lime was mixed with the coal and clay in groups D, E, F. The proportion between coal and clay in groups A, B and C was 7:3, and the proportion of coal, clay and lime in groups D, E and F was 7:1.5:1.5. Only block coal which was not mixed with the clay was used in group G. The results showed that the average fluorine content of the corn dried by coal mixed with clay was notably higher than that dried by block coal only. The difference of average fluoride content between the dry corn dried by coal mixed with M clay and that by coal mixed with T clay was not notable, but the average fluorine content in the dry corn dried by coal mixed with P clay was notably higher than that by coal mixed with M clay and T clay. The results also show that mixing the lime with coal and clay can effectively decrease nearly 90% of SO₂ pollution when they were burnt. It was noticeable that the average fluoride content of the corn dried by coal mixed with T clay and a special Ordovician lime was almost equal to that dried by block coal only. Clay is a major fluorine source for fluorosis in the coal-burning endemic fluorosis area in southwest China. This work is supported by the National High-Tech R & D Program (863 Program) (2004AA601080, 2006AA06Z380) and

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Key word: China, Southwestern; Fluorosis areas; Indoor coal-combustion; Sulphur.

GEOCHEMISTRY OF FLUORINE IN CHILIES FROM SOUTHWESTERN CHINA AND THE IMPACT ON PUBLIC HEALTH

Abstract number:

45

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Conference

The purpose of this research was to investigate the fluoride content of fresh chilies and dried chilies in Southwest China; the distribution of fluoride levels in chili; and the application of the results to calculate the difference of fluoride levels in the fresh and dried chilies so as to apply the outcome for the prevention of the fluoride contamination in chili.

In the research, the method for analysis of fluoride in food (GB/T 5009.18-2003) was applied to determinate the fluoride content in chilies. The average fluoride level in chilies was obtained by analysis of about 176 fresh chili samples and 296 dried chili samples collected from 77 counties in Southwest China. Based on the differences of chili producing areas, chili variety, dehydration method, storage time and fluoride forms, a systemic statistics of the fluoride contents in chilies was established. The geometric mean of fluorine content in the fresh chilies was up to 8.9mg·kg⁻¹ (dry weight, 176 samples, confidence level: 95%). The geometric mean of the fluorine content in the dried chilies was 19.6mg·kg⁻¹ (dry weight, 296 samples, confidence level: 95%). The results showed that an inappropriate dehydration method and the storage time were the two main reasons leading to fluorine contamination in chilies. The results of studying the fluorine content of fresh chilies show that the fluorine content standard of vegetables in GB2762-2005 is inappropriate for chilies and we recommend 24.7 mg/kg (dry weight) and 5.2 mg/kg (fresh weight) as the fluorine contaminated discrimination values for fresh chilies. The project was supported by National Basic Research Program of China (2006CB708513) and the Bureau of Science and Technology of Guizhou province.

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Keywords: Chilies; Coal; Endemic fluorosis; Fluorine pollution.

STUDY ON BRICK TEA TYPE FLUORIDE-ALUMINUM COMBINED TOXICOSIS IN HULUNBEIER, INNER MONGOLIA, CHINA

Abstract number:

P1

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The objective of this study is to discuss whether fluoride-aluminum combined toxicity exists in habitants of HulunBeiEr, Inner Mongolia who drink brick tea with a high level of fluoride. Fluoride and aluminum were measured in drinking water, brick tea, milk tea, staple food, vegetable, and meat, as well as in the herdsmen's hair, serum and urine. A clinical examination was made and skeletal X-rays were taken. The blood of herdsmen,

living in the epidemic area of fluorosis caused by drinking brick tea, was analyzed by biochemical examinations. Persons rarely drinking brick tea and others suffering from high fluoride drinking water fluorosis in the same area were the control groups. In the brick tea fluorosis affected area, the fluoride and aluminum concentrations of water and food samples were at a normal level. Brick tea contained a large amount of fluoride at 541.3±48.8 mg/kg and aluminum at 4351±724 mg/kg. The fluoride and aluminum levels in milk-tea were 3.83±0.73 mg/L and 4.28±1.56 mg/L, respectively. In the high fluoride drinking water affected area, fluoride and aluminum levels in drinking water were 3.68±1.05 mg/L and 0.45±0.25 mg/L respectively; Brick tea accounted for 96.15% of total amounts of fluoride intake, and contributed to 89.60% of total aluminum intake in brick tea fluorosis affected area; The incidence of dental fluorosis in children was 25.24% in the brick tea fluorosis affected area, which was significantly lower than that (63.17%) of the high fluoride drinking water affected area (p<0.01). The incidence of adult fluorosis was 42.61% in the brick tea fluorosis affected area which was markedly higher than that (28.83%) of the high fluoride drinking water affected area (p<0.01). The incidence rate of epiphysis articular surface sclerosis of young patients suffering from forepart skeletal fluorosis was 26.7% in the brick tea fluorosis affected area, and 11.0% in the high fluoride drinking water affected area (p<0.01). The incidence rate of adult skeletal fluorosis was 65.7% in the brick tea fluorosis affected area, and 63.3% in the high fluoride drinking water affected area; The incidence rate of IIIrd degree skeletal fluorosis was 0.95% in the brick tea fluorosis affected area, and 5.50% in the high fluoride drinking water affected area (p<0.05). The incidence rates of osteopetrosis, osteoporosis, arthrosis degeneration and periosteal ossification were 12.86%, 35.27%, 66.19%, 35.24% in the brick tea fluorosis affected area respectively, and those in the high fluoride drinking water affected area were 30.27%, 6.42%, 40.37% and 19.27% (p<0.01). The fluoride content of the herdsmen's serum and urine were 0.10±0.07 mg/L and 2.41±1.29 mg/L in the brick tea fluorosis affected area respectively, and those in the control group were 0.04±0.02 mg/L and 0.72±0.38 mg/L (p<0.01). The aluminum content of the herdsmen's hair, serum and urine were 23.97±8.96 mg/kg, 0.96±0.37 mg/L and 3.31±1.14 mg/L in the brick tea fluorosis affected area, and those in controls were 9.15±3.35 mg/kg, 0.19±0.11 mg/L and 2.14±0.99 mg/L (p<0.01). The contents of the herdsmen's serum Ca, CK, AST, HBDH, ALP in the brick tea fluorosis affected area were obviously higher than those of control (p<0.05). However, the serum BUN and CRE were lower (p<0.01). The pathogenesis of fluorosis by drinking high fluoride brick tea in HuLunBeiEr was complicated. Combined fluoride-aluminum toxicity may exist in this area.

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Keywords: Aluminum; Brick tea; Toxicity of fluoride and aluminum.

EXPERIMENTAL EVALUATION OF MUSCULAR DAMAGE IN FLUOROSIS

Abstract number:

P2

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The experiment was designed to investigate the pathological effects of fluoride on gastrocnemius muscles of rats. In this study, sodium fluoride was administered subcutaneously to male and female Sprague-Dawley albino rats for two to four weeks. The treatment with sodium fluoride caused severe alterations in the shape and structure of gastrocnemius muscle of rat of experimental Group II (30 mg NaF/kg bw/day), Group III (45 mg NaF/kg bw/day) Group IV (75 mg NaF/kg bw/day) dosed for two weeks, and Group V (30 mg NaF/kg bw/day) Group VI (45 mg NaF/kg bw/day), and Group VII (75 mg NaF/kg bw/day) treated for four weeks, as compared to control Group I. In Group II and V, the pathological changes in the form of atrophy, hypertrophy, vacuole formation, retraction from perimysial sheath, disintegration and hypertrifurcation of muscle fiber were observed. In Group III and VI, disorganization of muscle fibers, severe atrophy, hypertrophy, nuclear hyperplasia, pyknosis, and formation of pyknotic nuclei towards periphery of muscle fibers were noticed. In the remaining higher dose Groups IV and VII, there was increased nuclear hyperplasia, focal necrotic areas, fascicular atrophy and complete disintegration of muscle fiber.

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Keywords: Gastrocnemius muscle; Histopathology.

CEREBRAL NEURODEGENERATION IN EXPERIMENTAL FLUOROSIS

Abstract number:

P3

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Sixty young male and female Sprague Dawley albino rats were divided into 7 groups, one control and 6 experimental. The control rats were injected with double distilled water 1cc/kg bw/day. The experimental groups were treated with 30, 45, and 75 mg NaF/kg bw/day respectively for 20 days and 35 days. The animals were sacrificed and the cerebrum was analysed for neurodegenerative anomalies. The edematous foci were formed in the cerebral cortex containing degenerating glial cells. The glial cells became vacuolated and showed hyperchromatization of nuclei in brain of rats of experimental group V. An elongated highly chromatolytic region with large number of vacuolated cells was visible. In some neurons, neuroplasm became hyperchromatic, with prominent fragmentation and apoptosis of nuclei. Pleomorphic, irregular glial cells showed necrosis. The cerebral cortex exhibited diffused haemorrhages. The chain formation of the disintegrated glial cells and large globose shaped neurifibrillary tangle inside the perikaryon in cerebral cortex were observed in rats treated with 45 mg NaF/kg bw/day for 35 days.

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Keywords: Albino rats; Cerebrum; Glial cells.

EFFECTS OF SODIUM FLUORIDE ON MMP-13 mRNA AND TIMP-1 mRNA IN RAT BONE TISSUE

Abstract number:

P4

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Conference

The objectives of this study were to observe the expressions of MMP-13 mRNA and TIMP-1 mRNA and to analyse the molecular mechanism of bone matrix degradation in the progress of rat subchronic fluorosis. Male Wistar rats were randomly divided into two groups according to body weight, i.e., a sodium fluoride group and a normal control group. The sodium fluoride group was given drinking water with 150 mg/L sodium fluoride, and the normal control group was given only tap water. The animals were bred for 24 weeks. Every four weeks some rats were killed. The contents of serum fluorine and bone fluorine were examined and analyzed. The changes in the osteoclasts were observed by routine transmission electron microscopy. The expression levels of MMP-13 mRNA and TIMP-1 mRNA were analyzed by RT-PCR. The fluoride concentrations of serum and femurs were found to be significantly increased in NaF-treated rats after four weeks, and the bone fluoride levels increased every month. The rat fluorosis model was established. The number of lysosomes and synthesis of lysosome enzymes in the osteoclasts were decreased. The expression levels of MMP-13 mRNA and TIMP-1 mRNA were also significantly increased in NaF-treated rats after four weeks ($p < 0.05$), and remained high until 24 weeks. High dose fluoride might persistently induce the expressions of MMP-13 mRNA and TIMP-1 mRNA, regulate the activity of osteoblasts and osteoclasts, and finally influence bone turnover.

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Keywords: Bone turnover; Fluorosis; MMP-13; RT-PCR; TIMP-1.

EFFECT OF SUBCHRONIC FLUOROSIS ON iNOS EXPRESSION IN RAT BONE TISSUE

Abstract number:

P5

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Conference

The objectives of this study were to observe the expressions of inducible nitric oxide synthase (iNOS) in the progress of rat subchronic fluorosis, and to analyse the association between NO free radical injury in bone and subchronic fluorosis. Male Wistar rats were randomly divided into two groups according to body weight, i.e., a sodium fluoride group and a control group. The sodium fluoride group was given drinking water having 150 mg/L sodium fluoride, and the control group was given tap water only. The rats were bred for 24 weeks. Every four weeks some rats were killed. The serum fluorine and bone fluorine were examined and analyzed. The levels of serum NO were quantified by Griess Reagent. The expression of iNOS mRNA and protein were analyzed by RT-PCR and immunohistochemistry. The rat fluorosis model was established. The expression levels of MMP-13 mRNA and TIMP-1 mRNA were also significantly increased in NaF-treated rats after four weeks

($p < 0.05$), and remained high until 24 weeks. The serum NO contents significantly increased in the NaF-treated rats after 8 weeks, but significantly decreased after 20 weeks and 24 weeks. The expression of iNOS mRNA significantly increased in rats treated with sodium fluoride and these proteins mainly appeared in hyperplastic zone and hypertrophic zone cells of epiphyseal plate cartilages, articular cartilage cells, osteoblasts and ligament cells. High dose fluoride might persistently induce the expressions of iNOS, and catalyze synthesis of NO, regulate osteoblast activity and osteoclast activity, and finally influence bone turnover.

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Keywords: Bone turnover, Fluorosis; Immunohistochemistry; iNOS, RT-PCR.

EFFECTS OF SODIUM FLUORIDE ON LIPID PEROXIDATION AND ANTIOXIDANT ENZYMES IN SUBMANDIBULAR GLANDS OF RATS

Abstract number:

P6

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Fluoride is widely regarded as the cornerstone of modern preventive dentistry. Throughout the years, excessive intake of fluoride has become a serious health problem and, for that reason, there is a continuing concern about its toxicity and deleterious effects. Recent studies have demonstrated its effect of interfering with the antioxidant defense system. The purpose of this investigation was to evaluate acute sodium fluoride toxicity on some antioxidant enzymes and lipid peroxidation in the salivary submandibular glands of rats. Wistar male rats were separated in two groups: the experimental group was treated with sodium fluoride solution (NaF) intraperitoneally in a dose of 15 mg F⁻/kg of body weight and, the control group was administered the same volume of sodium chloride solution (NaCl 3.05%). The animals were euthanized 0, 1, 3, 6, 12 and 24 hours after the injection and the submandibular glands were removed and assessed for the activity of catalase (CAT) by the Aebi method (1984), and superoxide dismutase (SOD) by the Paoletti and Mocali method (1990). Lipid peroxidation level was measured by the method of Esterbauer and Cheeseman (1990). Protein content was determined by the method of Lowry *et al.* (1951). Statistical analysis was performed using analysis of variance and Tukey test at 5% significance level ($p < 0.05$). Changes in the enzymes activities were observed after the fluoride treatment in an exposure-time dependent manner. When compared to the control group, there was a significant increase in SOD activity of 45.67% after 3 hours and a decrease of 39.79% after 24 hours of NaF administration ($p < 0.05$). Conversely, the CAT activity was higher after 3 (41.29%), 6 (30.21%), 12 (60.11%) and 24 (49.80%) hours of exposure to NaF as compared to control animals ($p < 0.01$). Lipid peroxidation levels, measured by the malondialdehyde (MDA) content, were enhanced after 3 (54.67%), 6 (176.88%), 12 (166.88%) and 24 (141.18%) hours of fluoride exposure ($p < 0.01$). There was no significant difference in the protein concentration. These findings suggest that fluoride altered the free radical scavenger activity and enhanced lipid peroxidation, thus indicating its involvement in oxidative stress of the submandibular glands in rats.

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Keywords: Antioxidant enzymes; Lipid peroxidation; Oxidative stress; Salivary submandibular glands.

TOTAL ANTIOXIDATIVE STATUS OF ISOLATED HEPATOCYTES EXPOSED TO F⁻ ION ACTIVITY

Abstract number:

P7

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Current reports concerning the explanation of the role of fluoride ions in causing oxidation stress in the cell are incomplete. It is unclear, for example, whether the activity of fluoride ions disturbs the balance between pro- and antioxidants. Therefore the aim of this work was to assess the possibility of restoring hepatocyte oxidative homeostasis, disturbed by F⁻ ions, through the use of N-acetylcysteine (NAC). This was to be achieved by measuring the value of total antioxidation status (TAS). For this study, hepatocytes were sampled from Wistar rats by means of an enzymatic method. The isolated hepatocytes were incubated for 10, 30 and 60 min in L-15 medium containing F⁻ ions at a concentration of 0.082 mmol/L and/or NAC at a concentration of 1 mmol/L and in pure medium (control trials). After incubation the cells were centrifuged and mechanically homogenized. In the homogenate we marked the total antioxidation status by means of Randox test set. The protein was marked by means of Lowry's method (1975). The TAS value was expressed in μmol converted to mg of cell protein. It was established that the TAS value decreased significantly within the time span of the experiment. After fluoride incubation periods of 10 minutes and 30 minutes, the TAS value measured decreased by about 10% in comparison with the control sample. Prolonging the incubation time to 60 minutes caused the TAS value to decrease by a further 20% in comparison with the value measured in the control hepatocytes. This is certainly the consequence of the lowering of the concentration of hydrophilic antioxidants present in hepatocytes. Introducing NAC to the incubation mixture, together with fluorides, significantly increased the TAS value. It increased by about 10–14% after 10 and 30 minutes of incubation, and after 60 minutes by about 22%, in comparison with the value measured in hepatocytes incubated solely with fluoride ions. Thus, the toxicity of fluoride ions probably results in the lowering of glutathione concentration, and the protective role of NAC is connected with its part in GSH renewal.

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Keywords: N-acetylcysteine; Oxidative stress; Rat hepatocytes; Total antioxidant status.

A COMPUTATIONAL STUDY ON A HYPOTHESIS LINKING ALUMINUM FLUORIDE TO ALZHEIMER DISEASE — SEARCHING FOR BINDING DOMAINS OF β -AMYLOID

Abstract number: **P8**
XXVIIth ISFR Conference

Aluminum fluoride is a neurotoxic compound which undergoes pH dependent dissociation into F^- and Al^{3+} ions. These ions may pass the blood-brain barrier (BBB) causing the self-aggregation of beta-amyloid (senile plaques) as observed in Alzheimer's (AD) and other forms of dementia. Indeed, many scientific reports have shown the potential role of AlF_x in the etiology of AD. Neurotoxic effects of the fluoride ion have been reported following investigations concerning fluoride accumulation in the brain (especially in the hippocampal structure) Aluminum has also been known to be a neurotoxic agent with a variety of data linking Al to AD. It has been proven that aluminum ions (next to Cu, Zn and Fe ions) are activate the self-aggregation of the β -amyloid precursor protein and other amyloid peptides. Metal ions may act as a chelators changing the β -sheet conformation of amyloid into α -helical one. Within this report we identify, using the methods of computational quantum chemistry, those amino acid chains occurring in β -amyloid that are prone to strong interactions with the $[Al(H_2O)_6]^{3+}$ ions. The gas phase and aqueous binding affinities for several model compounds, mimicking amino acid side chains present in β -amyloid, were obtained at the B3LYP level of theory employing the 6-31G(d) and 6-311+G(2df,2p) polarized basis sets of double- and triple- ζ quality, respectively. The self-consistent reaction field method was used to model solvent effects. Our computational study demonstrated that in aqueous solution the aluminum ions occur as an octahedral complexes solvated by six water molecules and preferentially bind to aspartic acid (Asp7 and Asp 23) and histidine (His14) residues. It was indicated by the free energies of the exchange reaction with the replacement one water ligand in $[Al(H_2O)_6]^{3+}$ with an amino acid side chain.

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Keywords: Aluminum fluoride; Alzheimer disease; β -amyloid amino acids; 6-coordinate aluminum complexes.

FLUORIDE INFLUENCE ON PREVALENCE OF OSTEOSARCOMA IN WESTERN POMERANIA BETWEEN 1980 AND 2006

Abstract number: **P9**
XXVIIth ISFR Conference

The treatment of primitive malignant bone tumors such as osteosarcoma has radically changed in recent years since modern imaging, reconstructive surgery techniques and all pre- and postoperative chemotherapy protocols were put into use. The prognosis and quality of life were improved significantly, however, the pathogenesis and etiology are still unclear. Several chemical agents such as beryllium, some viral infections, radiation, trauma and burn have been reported to be potentially associated with neoplastic initiation of osteosarcoma. Furthermore, for over thirty years scientists in many centers all over the world have attempted to find a causal nexus between increased amount of fluoride and more frequent occurrence of osteosarcoma in various populations. The proposed mechanisms of neoplastic initiation in osteosarcoma related to fluoride were based on the hypothesis of genome damage preceding the development of neoplasm. It was assumed that fluoride environmental overabundance as a potential mutagen could affect cellular genetic material and subsequently, leading to its mutation, being the reason for neoplastic initiation. Moreover, it was emphasized that fluoride acted as an artificial osteoblasts growth stimulator. Nowadays, most scientific reports do not confirm the hypothesis of the influence of increased fluoride concentrations on the higher prevalence of osteosarcoma in populations. The authors discuss the possibilities of scientific, clinical, and epidemiologic evaluation of the influence of fluoride on the occurrence of osteosarcoma in population of Western Pomerania Region.

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Keywords: Osteosarcoma; Western Pomerania.

THE INFLUENCE OF LIPID COMPOUNDS ON THE CONCENTRATION OF FLUORIDES IN THE ANTLERS AND BONES OF ROE DEER (*CAPREOLUS CAPREOLUS*)

Abstract number: **P10**
XXVIIth ISFR Conference

As fluorine is a lipophilic element, lipids play a significant role in the accumulation and metabolism of fluorides in inner organs and bodily fluids. One of the sites of interaction between the fluorides and lipids are the bones and antlers of roe deer. Although the general content of fluorides and lipid compounds has been well researched in bones, we know very little about the their mutual influence in the antlers shed by roe deer each year. Hence, the aim of this paper was to determine (using the STATISTICA Neural Networks software) the relationship between the content of total lipids together with the five most important fatty acids, (linoleic acid (C 18:2 Δ 9, 12), oleic (C 18:1, Δ 9), palmitic (C 16:0), stearic (C 18:0) and eicosadienic acid (C 20:2, Δ 11, 14), and the concentration of fluorides in the skulls and antlers of roe deer (*Capreolus capreolus* L.). The concentrations of fluorides were determined using ion selective electrodes, concentrations of total lipids using spectrophotometry, and the concentrations of fatty acids with gas chromatography using an internal standard (heptadecanoic acid C 17:0). Mean concentration of fluorides in the examined antlers was 0.3593 ppm (0.0004 mg/g; SD: 0.10718), and in the skull bones 0.3900 ppm (0.0004 mg/g; SD: 0.14988). The mean concentration of lipids in the antlers was 64.63736 mg/g (SD: 17.62648) and in the skull bones 73.03208 (SD: 22.69000). Among the fatty acids determined in this study, palmitic acid and stearic acid had the greatest differences between the concentrations

in bones and antlers. After parameter analysis (involving the place of living, age, individual characteristics, total lipids and the concentration of fat acids), it was found that in the antlers the most important parameter influencing the fluoride concentration was the total lipids, and in the bones these were the individual characteristics of the examined animals.

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Keywords: Lipids; Roe deer; Statistica neural networks.

EXCESSIVE FLUORIDE ACCUMULATION IN ANTELOPE MANAGED WITHIN A RESTRICTED GEOGRAPHIC AREA

Abstract number:

P11

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Conference

Fluoride is a halogen, estimated as the 13th most abundant element in the earth's crust. It is widely distributed in nature (rocks, soils, water, vegetation and animals) and, because of its high reactivity, fluorine occurs predominantly as inorganic fluoride compounds. Fluoride may also enter the environment as a pollutant and accumulate in vegetation by direct deposition from the atmosphere and uptake through plant leaves, or via uptake through roots from soils within contaminated habitats. Excessive fluoride intake by animals can lead to acute poisoning while successive intake of moderate levels of fluoride may accumulate in animal tissue over time. Fluoride selectively accumulates in the mineralised tissues of the body and in mammals 99% of the body burden of fluoride is found in bones and teeth. The skeletal fluoride content of an individual therefore provides a cumulative measure of net fluoride over a lifetime. High concentrations of fluoride in bone are regarded as evidence of fluoride toxicity. Fluoride is phytotoxic, and at a mean concentration of 20 mg/kg, can negatively affect tissues of plants. The aim of this study was to determine whether fluoride found in varying concentrations in the soil and leaves within a study area in Gauteng, South Africa were reflected in bone tissue derived from resident blesbok and impala. Bone samples were collected over years as part of a routine sampling programme. Control samples of the same species were acquired off site. Fluoride levels in soil and leaves were determined at selected sites throughout the study area. The soil fluoride level had a mean of 52.8 mg/kg (< 5–1780 mg/kg), and the leaf fluoride levels had a mean fluoride of 51.1 mg/kg (< 20–1420 mg/kg). Control bone samples had a mean fluoride level of 1053 mg/kg (260–2020 mg/kg), and the bone samples collected within the study area had a mean fluoride level of 5673 mg/kg (4240–7520 mg/kg). Soil and leaf fluoride concentrations differed between the different localities sampled. The bone fluoride values in the study area samples significantly exceeded the controls collected at the three off-site locations. Measures have been put in place to decrease the release of fluoride into the environment at the study site and follow-up research will look at the effect which will keep eyes on the fluoride level in the resident antelope. This work will assist conservation management programmes where naturally high levels of fluoride are present for comparison within restricted environments and protected areas such as the Pilanesburg National Park.

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Keywords: Antelope; Restricted environment; Toxicity.

INVESTIGATION OF FLUORIDE CONCENTRATION IN DRINKING WATER SOURCES AND DMFT PREVALENCE AMONG 10 YEARS OLD SCHOOL CHILDREN IN SANANDAJ, IRAN

Abstract number:

P12

XXVIIth ISFR
Conference

The aim of this study was to estimate DMFT index (Decayed, Missing and Filled Teeth) in the 10-year-old students in Sanandaj city and to determine the fluoride concentration in drinking water Sources. The survey was performed using the cross-sectional method on 260 students selected using multi-stage sampling. The DMFT index of permanent teeth was determined, using the standard methods recommended by world Health organization. The water fluoride level was estimated in water supplies, using SPADNS method. The Annual Mean Maximum Temperature (AMMT) recorded during the last two years were Collected from the meteorological Center. A total number of 260 students (50% boys and 50% girls) aged 10 years and 200 water samples were assessed. The results showed that mean fluoride concentration of drinking water during one year was 0.4 ppm, which was less than normal level. The average AMMT of Sanandaj city is 18±4°C at which the optimal fluoride in drinking water of Sanandaj using Galagan and Vermillion equation was calculated to be 1 ppm. The mean DMFT value was 1.18±0.11 and was higher in girls in comparison with boys. The results indicate that the availability of other sources of fluoride must also be considered and taken into account in the planning of programs in public health dentistry.

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Keywords: DMFT; Fluoride in drinking water.

EFFECT OF FLUORIDE ON ACTIVITIES OF ENZYME AND ULTRASTRUCTURE IN PRIMARY CULTURED RAT HEPATOCYTES

Abstract number:

P13

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Conference

The purpose of this research was to study the cell viability, activities of enzyme and ultrastructure changes induced by sodium fluoride in primary cultured rat hepatocytes. Hepatocytes were isolated using half-*in situ* collagenase digestion method. Cellular viability was determined by MTT method. The activities of ALT and AST were determined by the spectrophotography method. The ultrastructure changes of hepatocyte were observed under transmission electron microscope. After being cultured with various concentrations of fluoride for 24 hours, a dose-dependent decrease of cell viability was detected in the hepatocytes. The activities of AST and ALT in the 2 mmol/L and 4 mmol/L groups were significantly higher than those of the control group ($p < 0.01$). Transmission electron microscope study showed that in fluoride treated hepatocytes the changes included swollen mitochondria and disordered, disrupted endoplasmic reticulum. Excessive fluoride induced significant toxicity in primary cultured hepatocytes and was manifested by injuries to the membrane and organelle plasma membrane.

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Keywords: Hepatocytes; Primary culture.

AMINO ACID METABOLISM AND EFFECTS OF GLUCOSE INFUSION IN FLUOROACETATE- EXPOSED RATS

Abstract number:

P14

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Conference

We analyzed amino acid metabolism and the effects of glucose infusion in monofluoroacetate (MFA) exposed-rats. There have been many cases of accidental or intentional MFA exposure. However, the mechanism of MFA toxicity has not been fully elucidated. It has been reported that fluoroacetate is converted to fluorocitrate, which then inhibits aconitase, a key enzyme in the tricarboxylic acid cycle, resulting in accumulation of citrate and lactate in the blood. Antidotal and clinical medications against MFA toxicity have not been put to practical use. There are no reports on the cause of MFA toxicity with regard to amino acid and amphibolic intermediate metabolism. This study was designed to elucidate the detailed metabolism of glucose, amino acids, and their related amphibolic intermediates in MFA-treated rats by measuring the concentrations of citrate, pyruvate, lactate, amino acids, and related agents. To evaluate the effect of glucose infusion on survival, rats were divided into two groups (a group treated with MFA plus saline and a group treated with MFA plus 10% glucose), and survival rates were determined. To analyze glucose and amino acid metabolism, additional rats were divided into three groups (a control group, a group treated with MFA plus saline, and a group treated with MFA plus 10% glucose), and blood samples were examined. The results showed that glucose infusion had no effect on the survival rate, but did increase the serum citrate and blood lactate concentrations. Interestingly, MFA significantly increased the concentrations of some of amino acids, and glucose infusion suppressed these increases. Dynamic changes observed in the metabolism of amino acids in MFA-treated rats may be explained by changes in the concentrations of amphibolic intermediates. These findings indicate that amphibolic intermediates, particularly α -ketoglutarate, may play an important role in MFA detoxication.

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Keywords: α -ketoglutarate; Amino acids, Amphibolic intermediates; Glucose infusion; Monofluoroacetate.

EPIDEMIOLOGICAL INVESTIGATION OF DRINKING WATER FLUORINE POLLUTION IN LONGHANG COUNTRY OF QIANNAN GUIZHOU

Abstract number:

P15

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Conference

The purpose of this investigation is to find the exact area of drinking water type endemic fluorosis in Longchang County of China. Students in 4 schools (Zhongxing, Longjing, Tuanpo and Tuanyang) were examined for dental fluorosis. The fluoride concentrations in the headwater, tip water, and the urine of students were measured. The dental fluorosis prevalence of Zhongxing, Longjing, Tuanpo, and Tuanyang was 19.0%, 17.5%, 15.8% and 40.0%, respectively. The fluoride contents in headwater and tip water were all less than 1.00 mg/L. The fluoride contents in headwater, high place and tipwater of Fuquan prison were 4.03 mg/L, 1.81 mg/L, and 1.60 mg/L, respectively. The reason for the fluoride pollution in the drinking water of Fuquan prison, ranging from 1.60 to 4.03 mg/L is being investigated.

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Keywords: Drinking water; Epidemiology; Fluorine pollution.

FLUORIDE LEVELS IN VARIOUS TEA-BASED BEVERAGES OF JAPAN AND IN CHINESE TEA INFUSIONS

Abstract number:

P16

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Conference

We have studied on accelerated bone resorption in middle-aged women of Inner Mongolia, china, where drinking waters are high in fluoride concentration. Inhabitants are always drinking brick tea infusions which are very high in fluoride concentration in grassland area. In Japan, various tea-based beverages are widely available on market as refreshments made with drinking water, and it is easy to buy Chinese tea leaves by internet. In order to estimate the fluoride intake, we determined fluoride levels in tea-based beverages and in infusions of Chinese tea leaves. We collected 70 samples of tea-based beverages in bottles, cans and paper packs with different trade names or

manufacturers on market in Japan, and 50 samples of Chinese tea leaves from the internet market. Infusions of Chinese tea leaves were made using distilled water by methods of attached documents. Fluoride levels were measured by the ion-selective electrode method. In tea-based beverages, oolong tea (n=17) had the highest average of 1.12 mg/L (range, 0.76–1.62 mg/L), and the next highest average was green tea (n=27) of 0.96 mg/L (0.62–1.78 mg/L), (black) tea (n=7) of 0.70 mg/L (0.44–0.91 mg/L). We could not find fluoride in beverages made of barley and buckwheat. We could detect fluoride from blending tea beverages (n=6) for which tea leaves were used in part (0.23–2.04 mg/L). In infusions of Chinese tea, oolong tea (n=14) had the highest average at 1.23 mg/L (0.61–2.56 mg/L), and (black) tea (n=9) was next with 1.18 mg/L (0.53–2.12 mg/L), then green tea (n=6) with 0.86 mg/L (0.43–1.49 mg/L). Chinese black and pu-erh tea (n=14), the same kind of brick tea, had an average of 0.93 mg/L (0.39–2.25 mg/L). Although we found only a few samples in oolong tea, black tea and Chinese black tea infusions with fluoride levels as high as in brick tea infusions (2.61–10.9 mg/L) of Inner Mongolia, China, 70% of tea-based beverage samples and 60% of Chinese tea infusion samples were over the recommended limit level for fluoride in drinking water (0.8 mg/L). We suggest that the fluoride intake from tea-based beverages and infusions of china tea leaves is large, and that it is necessary to conduct more advanced research on fluoride hazards.

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Keywords: Fluoride levels in tea; Chinese tea infusion; Tea-base beverage of Japan.

DOES FLUORIDE EXPOSURE CAUSE ACCELERATED BONE RESORPTION IN INNER MONGOLIAN YOUNG WOMEN?

Abstract number:

P17

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Conference

We found accelerated bone resorption in the middle-aged premenopausal women in the grassland area of Inner Mongolia, China, where drinking waters are high in fluoride concentration, and it is common to drink brick tea infusions which have a very high in the fluoride concentration. In order to clarify the influence of fluoride exposure on bone mass and bone metabolism in young premenopausal women, we carried out a case control study.

We studied 37 young women, aged 20–34, in the grassland area, and 45 young women in urban area. Speed of sound (SOS), broadband ultrasound attenuation (BUA) and stiffness index (SI) were measured at the calcaneus using quantitative ultrasound (QUS) analysis. Bone mineral density (BMD) and metacarpal cortical index (MCI) of second bone of middle finger were measured by X-ray (CXD). Fluoride levels in drinking water, brick tea infusion and urine were measured using ion-selective electrode method. Bone alkaline phosphatase (BAP) in serum, CrossLaps (CTX), pyridinoline (PYD), deoxypyridinoline (DPD) and hydroxyproline (Hyp) in urine were measured as metabolism markers. The fluoride levels of the drinking water and brick tea infusions in grassland area were 2.43 mg/L, and 4.85 mg/L, respectively. The fluoride level of the urine (2.95 mg F/g creatinine) in grassland women was significantly ($p < 0.001$) higher than that of urban women (0.63 mg F/g creatinine). The bone mass index, SOS, SI, and MCI in the grassland women were significantly lower than those of urban women ($p < 0.01$, $p < 0.05$ and $p < 0.05$, respectively). Although the serum BAP, a bone formation marker, in grassland women was higher than that of urban women ($p < 0.05$), urinary bone resorption markers, CTX, PYD, DPX, and Hyp in grassland women were very higher than those of urban women ($p < 0.001$, respectively). Urinary bone resorption markers were significantly correlated with urinary fluoride level ($p < 0.01$, respectively). We found that a lower bone mass and an accelerated bone resorption in grassland women were associated with fluoride exposure, suggesting that fluoride exposure may cause accelerated bone resorption and osteoporosis but not skeletal fluorosis or osteosclerosis.

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Keywords: Accelerated bone resorption; Bone mass decrease; Drinking water and brick tea; Fluoride exposure; Young premenopausal women.

RELATIONSHIP BETWEEN DENTAL CARIES AND DRINKING WATER FLUORIDE AMONG CHILDREN IN ESTONIA

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P18

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Dental caries continues to be a public health problem in many countries. Great variations are seen between and within countries. Fluoride in drinking water is considered to play an important role in the prevention of dental caries. In recent years, the protective effect of drinking water fluoride has been questioned in some studies. The aim of this study was to investigate the relationship of fluoride level in drinking water and caries experience among

3-years old children in Estonia. The study was conducted in six towns relying on local groundwater sources with different fluoride concentrations. The towns were of approximately the same size, socioeconomic standards and ecological situation. Samples of drinking water were collected from public water supply systems in these towns. Fluoride concentration in drinking water was measured by SPADNS method. The study subjects were selected from a survey of cross-sectional clinical examinations of children attending kindergartens. The dental health of children was investigated according to WHO unified methodology. In total 341 children (mean age 41 months) were included into the study. In further analysis 3 indicators of caries experience were used: prevalence (%), dmft-score and SiC-index. The fluoride concentration in drinking water of 6 towns ranged from

0.25 to 1.10 mg/L; caries prevalence ranged from 34.1–56.3%, mean dmft-score 1.06–2.31 and SiC-index 3.18–5.45. There was a strong negative correlation between fluoride content in drinking water and all caries indicators (Pearson correlation coefficient –0.89, –0.95 and –0.99 for prevalence, dmft-score and SiC-index, respectively). The study showed that natural fluoride in drinking water has a beneficial effect against caries. The strongest correlation was found with SiC-index which demonstrates more clearly the protective effect of fluoride in drinking water in severe cases of caries.

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Keywords: Dental caries; Fluoride in drinking water; Estonia.

PREVENTION ON STAINING AND PITTING OF CHILDREN'S DENTAL FLUOROSIS IN HIGH-FLUORIDE AREA OF SHANXI PROVINCE

Abstract number:

P19

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Children who continuously lived in high-fluoride areas in their early childhood are inevitably suffered from dental fluorosis when their permanent teeth erupted. Consequently, staining and pitting of enamel affected esthetic and quality problems in dental fluorosis. The purposes of the present study were: 1) whether we can prevent the extrinsic staining and subsequent posteruptive damage by artificial remineralization; 2) to determine the duration of the 'posteruptive maturation' process of enamel fluorosis. We selected 106 study subjects of 7-12 year-old children from the primary schools in high-fluoride areas having fluoride concentration ranging from 3.0 to 3.2 ppm in the drinking water. The left upper incisor is covered with artificial mineralization film and right upper incisor covered by water as self-control every three months. The treatment period was 24 months from June, 1997 to June, 1999. The color change of the upper incisors was checked and recorded by taking pictures in the 24th, 34th, and the 82nd month after stopping the treatment. In the 24th months, we found that the process of staining had stopped or the degree of staining was reduced after 9 months of treatment. During the 24 months, we also found that the color of control side had turned into black. In most of cases, the degree of staining had increased mitigation, compared to the previous situation. The color of the control side had darkened significantly in the 34th and the 82nd month after stopping the treatment. Interestingly, we discovered pitting of enamel fluorosis had also stopped. The clinical study showed that the mineralization film had a function of artificial remineralisation and anti-permeated the extrinsic staining into the enamel fluorosis. The mineralization film could stop pitting of fluorosis enamel. The posteruptive maturation process of enamel was uncompleted even though the children had reached the age of 16.

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Keywords: Enamel fluorosis; Pitting; Posteruptive maturation; Staining.

THE 100 YEAR HISTORY OF INDUSTRIAL FLUORIDE POLLUTION AND FLUOROSIS

Abstract number:

P20

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Conference

The history of industrial fluoride pollution dates back to 1855, when there was a case of compensation for loss for cattle farms polluted from exhaust fumes from German smelter industries. However, it was not until 1907 that final confirmation was obtained that the toxic chemical in the smelter smoke that poisoned the cattle was fluoride. In December 1930, a pollution disaster in Meuse Valley, Belgium, occurred due to fluoride. Early in the 1930s, a Danish scholar, Professor P. Flemming Moller first reported the changes of bone shown on the X-ray films of the cryolite workers, and suspected that they were caused by fluoride. In 1932, he named this disease as fluorosis. Subsequently, Doctor Kaj E Roholm endorsed and confirmed the harmful effects of fluoride on the cryolite workers and published his famous monograph *Fluorine Intoxication*. Christopher Bryson reviewed the related literature, including classified and official documents from the time of World War II, and obtained personal information from generals in the armed forces. His book, *Fluoride Deception*, reveals the tragic story of an American fluoride-pollution incident, which was concealed for 60 years. He found that the Manhattan project led to serious fluoride pollution, as in order to obtain fissionable uranium-238 from stable uranium-235 a huge amount of UF₆ was used. During this period factories discharged high concentrations of fluoride which polluted the surrounding areas. The worst case, the Donora pollution disaster, in November 1948, was caused by fluoride. Bryson stated that the industrial groups contrived present the view that fluoride pollution was "safe" in order to protect the industries and that this helped to win hazard law suits involving industrial fluoride pollution. Krook and Maylin (1979) reported that, from 1973, fluoride pollution from an aluminum smelter on Cornwall Island in Canada severely affected dairy farm production. Subsequently, fluoride pollution affected dairy production in a nearby farm in New York state, USA. In China, industrial fluoride pollution was reported in 1960s. Fluorosis was first diagnosed in China in grazing lands around the industrial region in Baotou. Symptoms of severe dental fluorosis in herbivores included excessive tooth wear and bone lesion. Commonly, the molar teeth were worn down to the gum line. Exostoses developed on the cervical ribs. Dental lesions impaired the ability of the animals to forage and led to malnutrition, low productivity with infertility, miscarriages, and even death. An investigation in 1974 found that the area of endemic fluorosis extended up to 100,000 km². More than 6,000 fluorosis cases in animals were detected. As a result it has become less economic to farm cattle and horses. At present, the herdsman can only raise sheep and goats and the problem continues to exist.

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Keywords: Environmental fluoride; Fluorosis; History of fluoride pollution; Industrial pollution.

THE FORMATION AND ENRICHMENT REGULARITIES OF HIGH FLUORIDE IN GROUNDWATER IN DALI COUNTY, SHAANXI

Abstract number: **P21**
XXVIIth ISFR Conference
Investigations, including assessing climate, geology, morphology, hydrogeology, hydrogeochemistry, were made in order to clarify the formation and enrichment regularities of high fluoride in the groundwater in Dali County, Shaanxi. The results showed that the high fluoride in this groundwater was formed under the joint action of filtration, accumulation, vaporization, and concentration in specific hydrogeochemical conditions.

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Keywords: Enrichment regularities; High fluorine groundwater; Origin of fluoride in groundwater.

CHANGES IN THE CONCENTRATION OF F AND BIOGENIC ELEMENTS IN THE TEETH OF RATS WITH TYPE 1 DIABETES AS A FACTOR POTENTIALLY INDUCING TOOTH DECAY

Abstract number: **P22**
XXVIIth ISFR Conference
Tooth decay is a multi-factor disease, where hard tooth tissues are dissolved by bacterial acid products coming from decomposition of sugars with low particle mass. The mineral content of the tooth hard tissues may influence the speed of decay changes; it is known that the fluorine ions are capable of stabilizing the structure of hydroxyapatite, the main building material of teeth, and in this way decrease its solubility and increase resistance to

decay. However, a high supply of fluorine may be a factor disrupting the mineralisation of tooth tissues. Magnesium stabilizes amorphous calcium phosphate and inhibits the formation of crystalline hydroxyapatite, the main building material of hard tooth tissues. Considering these facts, we aimed to examine if type 1 diabetes may be a factor contributing to the appearance of tooth decay. The study involved female Wistar rats, which were given 1 mL streptozocine in a dose of 55 mg/kg body weight in 1 mL 0.01 M citrate buffer (pH 4.5), to induce diabetes. Control group was given 1 mL 0.01 M citrate buffer with pH 4.5. Diabetes was recognized when glucose concentration in whole blood >11.0 mM. After 10 days, the teeth and blood serum were taken from the animals. Fluoride concentration was determined with the potentiometer method; calcium and magnesium concentrations were determined with the atomic absorption spectrometry. In rats with type 1 diabetes we observed a statistically significant decrease ($p = 0.026$) in fluorine concentration in teeth compared with control. The concentration of fluorides in the blood serum of rats with type 1 diabetes was lower than control, but the difference was not statistically significant. The calcium concentration in the teeth of the control group and the study group was similar, but in the serum of diabetic rats it was significantly higher ($p = 0.000009$) versus control. The concentration of magnesium in the teeth of rats with type 1 diabetes was significantly higher ($p = 0.014$) than control. In the serum similar relationship was observed, but it did not reach the assumed level of statistical significance ($p = 0.06$). The results indicate that type 1 diabetes may be a potential decay-inducing factor.

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Keywords: Rats with type 1 diabetes; Serum; Teeth; Tooth decay.

CHANGES IN THE CONCENTRATION OF BIOGENIC ELEMENTS IN THE BLOOD SERUM AND BONES OF RATS WITH TYPE 1 DIABETES, AND THEIR POTENTIAL ROLE IN THE APPEARANCE OF OSTEOPOROSIS

Abstract number: **P23**
XXVIIth ISFR Conference
Diabetes is a group of metabolic diseases, characterized by hyperglycemia resulting from disruption in the secretion of and (or) action of insulin. Chronic hyperglycemia in diabetes may lead to the impairment and damage of many organs. Chronic lack of insulin, characteristic for the type I diabetes, may decrease the production of osteoid matrix and the mineralisation of bone tissues. Moreover, the deficiency of insulin-like growth factors

(IGF-1, IGF-2), inhibitors of resorption and stimulators of the bone restoration, increases the bone atrophy. Considering these facts, we aimed to examine if diabetes may be a factor contributing to the appearance of osteoporosis. The study involved female Wistar rats, which were given 1 mL streptozocine in a dose of 55 mg/kg body weight in 1 mL 0.01 M citrate buffer (pH 4.5), to induce diabetes. Control group was given 1 mL 0.01 M citrate buffer with pH 4.5. Diabetes was recognized when glucose concentration in whole blood >11.0 mM. After 10 days, the thighbones and blood serum were taken from the animals. In the examined material, fluoride concentration was determined with the potentiometer method; calcium and magnesium concentrations were determined with the atomic absorption spectrometry. The results of the study were analyzed with Mann-Whitney U test. The significance level was $p = 0.05$. Diabetic rats were observed to have much higher concentration of fluorine in bones compared with control, but it did not qualify as significant within the assumed level of significance. The concentration of fluorides in the blood serum of diabetic rats was lower than in control (no statistical significance). The concentration of calcium in the bone of control group was insignificantly lower than in the diabetic group, but in the serum of rats with type 1 diabetes it was significantly higher ($p = 0.000009$) versus control. The concentration of magnesium in the bones of diabetic rats was significantly higher ($p = 0.009$) than control. In blood serum, similar relationship was observed, but the difference was not statistically significant ($p = 0.06$). The obtained results suggest that the chronic disease, such as diabetes, may change the content of elements in bones, which in turn can be an etiological factor in appearance of osteoporosis.

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Keywords: Bone; Osteoporosis; Rats with type 1 diabetes; Serum.

SURVEY OF FLUORIDE CONCENTRATION IN DRINKING WATER SOURCES AND PREVALENCE OF DMFT IN THE 12 YEARS OLD STUDENTS IN ZANJAN, IRAN

Abstract number:

P24

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This study was carried out to estimate the DMFT index (Decayed, Missing and Filled Teeth) in the 12-year-old students in Zanjan city and to determine the fluoride concentration in drinking water sources. The cross-sectional study used the standard dental indices dmft and DMFT for oral health assessment and was performed on 250 students selected using multi-stage sampling. The water fluoride level was estimated in water supplies, using the SPADNS method. The annual mean maximum temperature (AMMT) recorded during the last two years was obtained from the Zanjan Meteorological Center. A total number of 220 students (50% boys and 50% girls) aged 12 years and 60 water samples were assessed. A questionnaire was designed to record the status of the teeth. The results showed that mean fluoride concentration of drinking water during one year was 1 ppm, which was more than the normal level. The average AMMT of Zanjan city is 24.2±14°C at which temperature the optimal fluoride in drinking water of Zanjan, using the Galagan and Vermillion equation, was calculated to be 0.8 ppm. The mean DMFT value was 1.12±0.1 and was higher in girls in comparison with boys. The present study shows that under controlled health conditions with good education, an acceptable value for the DMFT can be obtained.

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Keywords: DMFT, Fluoride, Drinking water, Zanjan.

ANALYSIS REPORT ON THE SURVEY RESULT OF ENDEMIC DRINKING WATER FLUOROSIS IN QINGHAI IN 2006

Abstract number:

P25

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Conference

The purpose of this investigation is to study the prevalence of fluorosis in 7 counties of Qinghai province after improvements to the water supply. The water quality was improved in 105 villages accounting for 59.66% of the total of 176 villages with endemic fluorosis. Thirteen villages were found in which the fluoride concentration was still higher than 1.0 mg/L, accounting for 12.38% of a total of 105 villages. Among a total of 8316 children, 3869 (46.52%) had dental fluorosis. The findings indicated that fluorosis is still prevalent in the endemic fluorosis area of Qinghai province.

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Keywords: Dental fluorosis prevalence; Drinking-water fluorosis; Water supply improvements.

ANALYSIS OF MONITOR RESULTS OF WATER-RELATED ENDEMIC FLUOROSIS AREAS IN GUANGDONG PROVINCE FROM 1991 TO 2005

Abstract number:

P26

XXVIIth ISFR
Conference

The objectives of this study were to investigate the prevalence of endemic fluorosis after taking measures to provide for an improved fluoride-safe water supply to an endemic fluorosis areas in Fengshun county, Guangdong Province. Daizai village, Hupo village and Anquan village were identified as severe water-related endemic fluorosis areas, where the fluorosis situation had been continuously monitored for the past 15 years. The fluoride contents in the drinking water and urine of children were determined with the F-ion selective electrode method. The presence of dental fluorosis in children and adult skeletal fluorosis were examined for with Dean's index and X-ray, respectively. The prevalence rate of dental fluorosis of children fluctuated and decreased gradually. The dental fluorosis index in Daizai village, Hupo village and Anquan village decreased from 1.5, 3.0 and 1.3 to 0.79, 0.89 and 0.21 respectively. Urine fluoride concentrations for each age group of children in the three villages were all within the limit of 1.50mg/L from 2002. A gradual decrease was found in the prevalence rate for adult fluorosis and skeletal fluorosis, as detected by X-ray examination. After the provision of a fluoride-safe improved water supply to these endemic fluorosis areas, the total fluoride intake of the inhabitants decreased and the level of endemic fluorosis lessened. It is evident that the most effective method of preventing drinking water fluorosis is to provide an alternative fluoride-safe water supply. However, the proper management of improved water supply facilities should be strengthened, particularly the operation and maintenance of the system.

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Keywords: Control of endemic fluorosis; Fluorosis; Monitoring of fluorosis.

ANALYSIS OF THE PREVENTIVE RESULTS OF AN IMPROVED WATER SUPPLY THROUGH DEFLUORIDATION IN ENDEMIC FLUOROSIS AREAS

Abstract number:

P27

XXVIIth ISFR
Conference

The objectives of this study were to assess the preventive effects on endemic fluorosis of the provision of an improved water supply through defluoridation in endemic areas after several years of intervention. The outcomes of the study are to be used as the scientific basis for formulation of prevention and control strategies. Yanhu County, the severe

endemic fluorosis region in Shanxi province, was selected as the study area where prevention and control measure through the defluoridation of the water supply to the area had been implemented for some years. The investigation sites were selected by stratified and cluster sampling of study subjects in the endemic areas. Dental fluorosis of 8–12-year-old children and patients with serious skeletal fluorosis were examined in each site. Dental fluorosis in children was diagnosed by Dean's method recommended by the WHO. Patients with serious skeletal fluorosis were examined with "Diagnostic criteria of skeletal fluorosis (GB16396-1996)". The fluoride levels of drinking water and urine were determined by a fluoride-ion selective electrode. The fluoride concentrations in the defluoridated drinking water were found decreased significantly, after several years of intervention, in the endemic dental fluorosis and skeletal fluorosis areas ($P < 0.01$). However, the fluoride levels in the defluoridated drinking water in some endemic fluorosis areas were found to be still above the Chinese national drinking water standard. In every site of the endemic areas, detectable rates of dental fluorosis in children were above 40% and close to the middle prevalence. Patients with serious skeletal fluorosis were not found. In many regions with severe fluorosis, the degree of fluorosis severity has been improved and regions have been upgraded to slight fluorosis regions. However, the detectable rates of dental fluorosis in children varied significantly in the severe endemic area. Therefore, it was difficult to carry out prevention and control measures in the moderate and severe disease areas. The fluoride content in the children's urine was found to be higher in general than could be due to consumption of high fluoride water by children. Therefore, improvement of the drinking water quality is the most effective measure for the prevention and control of endemic fluorosis. In addition, it is necessary to ensure the proper management of the improved/defluoridated drinking water supply system, and its effective operation and maintenance as well as the control of the water quality.

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Keywords: Defluoridation of water; Effects of defluoridation of water; Endemic fluorosis; Water-improving.

MITIGATION OF FLUOROSIS IN CHILDREN THROUGH AMLA MURABBA

Abstract number:

P28

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A large number of Indians are forced to consume fluoride contaminated drinking water. A study was carried out to investigate dental fluoride prevalence in children and beneficial effect of Amla murabba ingestion. 20 children aged 6–12 years were selected from an endemic area (F^- 5.8 ppm). The subjects were given one piece of Amla murabba (weighing 100 gm) twice a day for one month duration. Dental fluorosis grading was scored according to Dean's index. Blood and urine samples were collected before and after treatment. Results were compared to control group consuming water with 1.4 ppm fluoride. Dental fluorosis was found to be moderate to severe and showed a slight decrease in severity after the treatment. In fluoride-exposed subjects, haematological parameters were elevated and almost restored to normal value after the respective treatment. Fluoride in serum as well as urine was significantly higher in subjects exposed to fluoride. Further, an increase in urine F level and decrease in serum F level was observed after the treatment. The serum SGOT, SGPT, and AIP levels were found to be increased significantly in subjects in the fluoride endemic area and were almost restored to the normal range after the treatment.

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Keywords: Amla, Dental fluorosis; Mitigation.

DEFLUORIDATION OF WATER WITH ALUM AND POLY-ALUMINUM CHLORIDE (PAC)

Abstract number:

P29

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Conference

Fluoride at very low concentrations is phytotoxic and harmful to livestock; and in man, excessive intakes of fluoride over many years may lead to a well-defined disorder—skeletal fluorosis. In addition, a number of recent studies have suggested that fluoride may be genotoxic. The usefulness of fluoride as a preventive against dental caries does not mean that high level exposure to the element should be tolerated. Contamination of the total environment with fluoride emissions and solid wastes from many industries is increasing. So removal of fluoride from high level fluoride water is important for the environmentalists. In this research defluoridation of water investigated with alum and PAC (Poly-Aluminum Chloride). Water with 4.14 ppm of fluoride was investigated with several concentrations of alum in a Jar-Test procedure. The optimum dosage of alum was 250 ppm and the optimum pH for high efficiency (97%) was 6.2. Water with 24.14 ppm of fluoride was investigated with several concentrations of PAC and the results indicated PAC with 1 ppm dosage (industrial grade) can remove fluoride with 51% efficiency at pH 7. In view of the economical aspects, PAC is better than alum in defluoridation. The results also showed alum efficiency is better than PAC with water with

a low level of fluoride and that PAC is better than alum for water with a high level of fluoride.

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Keywords: Alum; Defluoridation of water; PAC.

ANALYSIS OF REFERENCE CONCENTRATIONS OF URINARY FLUORIDE FROM JAPANESE INHABITANTS

Abstract number:

P30

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Conference

Urinary fluoride concentrations of healthy Japanese adults ($n=167$; 141 males, 26 females; mean age, 32 ± 9 years; age range, 19 to 59 years) were determined by Orion 96-09 BN combination fluoride ion selective electrode (FISE) connected to Orion 720Aplus bench top pH/ISE meters (Thermo Fisher Scientific, Inc., USA). By correcting the obtained

urinary fluoride concentrations for the specific gravity of 1.024, the histogram of urinary fluoride concentrations highly skewed towards low values with sharp peakedness (skewness=1.56, kurtosis=3.08). The normality of log-transformed histogram (skewness=0.12, kurtosis=0.07) and the straight line on log probability paper clearly showed a key feature of log-normal distribution fashion of urinary fluoride concentrations. The geometric mean (GM) of 613.8 μ g/l and 95% confidential interval (CI) of 241.0-1633.1 μ g/L were established for urinary fluoride reference values. As fluoride has a very short half-life in the body and the major route for fluoride excretion is via urine, human exposure is best measured by analysis of urine where the concentration is expected to be highest. The results of the GM and CI in this study will be useful as guidelines for the biological monitoring of fluoride in normal subjects and individuals at the risk of occupational or environmental fluoride exposure.

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Keywords: Ion selective electrode method; Log-normal distribution; Reference values; Trace elements.

RADIOLOGICAL MODIFICATIONS OF BONE AND JOINT AMONG RESIDENTS OF ENDEMIC FLUOROSIS AREA FOR IMPROVING WATER 40 YEARS

Abstract number:

P31

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Conference

The objectives of this study were to understand the effectiveness of control measures for endemic fluorosis, and the radiological manifestations of bone and joint disease, among residents of the endemic area over a long period of time after improving water. The radiographic signs of skeletal fluorosis were identified in two persons with skeletal fluorosis before the provision of the improved water commenced. X-ray radiographs were obtained of the pelvis, forearm, and (lower) leg of 28 selected residents, with and without bone and joint symptoms, after consuming the improved water for 40 years. The clinical symptoms of skeletal fluorosis and the presence of mottled teeth were investigated and the urinary fluoride content was measured. Radiographs of 2 patients who had skeletal fluorosis before improving the drinking water clearly showed III grade fluorosis with one patient (male) showing osteosclerosis and the other (female) osteomalacia. 15 out of the 28 study subjects had abnormal X-ray signs (53.6%) with 6 cases found of skeletal fluorosis (21.4%). The presence of III stage fluorosis was reduced. The changes in the joints were the most significant followed by bone changes while changes of in the periosteum were not evident. The urinary fluoride contents of the 28 study subjects were 0.99 \pm 1.52 mg/L, while fluoride level in the 6 skeletal fluorosis patients was found to be 1.40 mg/L. After 40 years of consuming improved drinking water, substantial amount of fluoride had been excreted from body, and severe skeletal fluorosis among the inhabitants has disappeared. However, the signs of bone and joint disease shown on the X-ray radiographs were still visible and the skeletal fluorosis present in some inhabitants prior to the improvements in the water supply also persisted. It is possible that a number of patients have experienced better health after consuming the improved drinking water for 40 years, but complete recovery may not be possible.

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Keywords: Bone; Defluoridation of water; Radiographs; Rehabilitation; Skeletal fluorosis.

A QUICK METHOD FOR DETERMINING TRACE AMOUNTS OF FLUORIDE USING ELISA

Abstract number:

P32

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Conference

The linearity response range for Fluoride-ion analyzed by Fluoride-ion selected electrode is between 3×10^{-6} – 1×10^{-1} mol/L. When the trace fluoride concentration at less than 0.06 μ g/ml in samples analyzed by a fluoride-ion electrode, the micro-volt E could not Nernst Respond with $\log C_{F^-}$, thus, the results of trace fluoride concentration in samples are unreliable Therefore, the development of a more economical and quicker method having high precision and accuracy is of great significance. In this study, the method of ELISA (Enzyme-Linked ImmunoSorbent Assay)-Alizarin complexone,3-aminomethyl,alizarin-N,N-diacetic acid was adopted using direct calibration curves when the fluoride-ion concentration was more than 0.01 μ g/mL. The linearity range of calibration curves was between 0.004–0.02 μ g and 0.05–0.30 μ g. The average of the correlation coefficient was 0.998–0.9991 and 0.9996–0.9999. For samples having a fluoride-ion concentration of less than 0.01 μ g/ml, we chose the method of calibration curves double standards addition. The linearity range of calibration curves was between 0.004–0.02 μ g. The average of the correlation coefficient was 0.998–0.9991. After determining the trace fluoride-ion concentration in some biologic samples including blood and tissue of humans and animals, some digestive tract fluids, plant material, and the atmosphere, we also determined its accuracy with linearity experiments and recovery rates by adding standard material into samples. At the same time we calculated its precision and sensitivity. Determining the trace fluoride-ion concentration in the digestive fluids of biologic and environmental samples can be more accurate by this method. In particular, the sensitivity is higher notably by the method of double standards addition. The limit of detection is 0.002 μ g. The rates of recovery are between 97.6%–100.1%, and the coefficient of variation is under 2.7%. The method described above is more economical and simpler. At the same time, the method takes less time than usual methods and its accuracy and precision are higher than for other methods.

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Keywords: Alizarin complexone; 3-aminomethyl; Detection limits; Elisa; Trace amounts of fluoride.

FLUORIDE AND DOWN SYNDROME — AN UPDATE

Abstract number:

P33

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Conference

Between 1956 and 1963, epidemiological investigations in the north central region of the United States revealed a significant positive association between the congenital malformation known as Down syndrome (DS, trisomy 21) and the fluoride (F) content of the mother's drinking water. These findings were subsequently confirmed by other studies in the 1970s in the eastern part of the U.S., including two that also found higher rates of DS births among younger mothers in higher F water areas. Although overlooked in a 2001 *BioMed Central* review, a detailed 1998 analysis (*Fluoride* 31/2:61–73) of the data in several of these studies authenticated their statistical significance. This presentation is an update of research reported by the author at ISFR XXI in Budapest in 1996 on the occurrence of DS in Lower Michigan during the years 1951–1964. In that work a 10 to 30 percent higher overall rate of DS births by maternal residence was found in urban areas with or after fluoridation of the municipal water supply. In cities of 25,000 or more inhabitants (1950 census) with 0.8–1.2 ppm F there were 235 white DS births (1.038 per 1000 live births), whereas in the nonfluoridated cities of this size there were 572 white DS births (0.920 per 1000 live births; $p < 0.065$ by chi-square). When the very large city of Detroit (not fluoridated until 1967) is excluded, the number of white DS births in the nonfluoridated group was 171, and the rate was 0.726 per 1000 live births ($p < 0.001$). A similar relationship was also observed for smaller communities of 2500 to 25,000 population. Likewise, among younger mothers, in agreement with the other U.S. studies, the rate of white DS births was higher in communities with 0.3–2.6 ppm F in the water supply than in those with =0.2 ppm F.

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Keywords: Down syndrome; Lower Michigan; Maternal age; Water fluoride.

INFLUENCE OF FLUOROSIS ON ERK1/2 SIGNAL TRANSDUCTION IN RAT BRAINS AND ON LEARNING AND MEMORY OF RATS

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P34

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Conference

The aim of the study was to investigate the changes of the Erk1/2 pathway in rat brains with fluorosis and the effects of fluoride on learning and memory of the rats. The SD rats were fed with different concentrations of fluoride (NaF) for six months to establish rat models with fluorosis. The activation of phospho-Erk1/2, phospho-Mek1/2, phospho-P90orsk, phospho-Elk and the expression of total-Erk in rat brains were detected by Western-blotting and immunohistochemistry. The spatial learning and memory ability were measured by the Morris water maze test. The results showed that in the group with a high amount of fluoride as compared to the controls, increased activation of phospho-Erk1/2, phospho-Mek1/2, phospho-P90orsk, phospho-Elk, as well as the expression of the total Erk1/2 were observed. The rats in the group with the higher-dose of fluoride displayed poor spatial learning and memory. The results indicate that long-term exposure of fluoride can activate the expression of the Erk1/2 neuronal signal pathway, which may be connected with the spatial accuracy and acquisition performance.

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Keywords: Erk1/2 pathway; Learning and memory; Morris water maze test; Western-blotting.

EFFECTS OF FLUORIDE ON LEARNING AND MEMORY OF RATS AND CHOLINESTERASE ACTIVITY IN RAT BRAINS

Abstract number:

P35

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Conference

In order to investigate the mechanism of brain disorder influenced by fluoride, learning and memory, cholinesterase activity were detected in rats fed with fluoride (NaF). The rats were randomly divided into 3 groups, e.g., normal control group (drinking water containing less than 1 mg/L of fluoride), lower fluoride exposure group (drinking water containing 5 mg/L of fluoride) and higher fluoride exposure group (drinking water containing 50 mg/L of fluoride). The rats were examined at the third month after feeding the various dose of fluoride. Learning and memory were analyzed by Morris Water Maze test. The results showed that as compared with the control group, the higher fluoride intake group showed a decrease in learning and memory, and activities of acetylcholinesterase and butylcholinesterase were inhibited in the brains of the rats. Significant changes in learning and memory, and the activities of acetylcholinesterase and butylcholinesterase in the rats' brains were not observed in the lower fluoride intake group. The results suggested that the mechanism for the decrease in learning and memory might be the decrease of the brain cholinesterase activity with a high fluoride intake.

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Keywords: Acetylcholinesterase; Butylcholinesterase; Fluorosis; Learning and memory.

CORRELATIONS BETWEEN FLUORINE, ARSENIC, AND IODINE IN 5 COUNTIES OF QINGHAI PROVINCE, CHINA

Abstract number:

P36

XXVIIth ISFR
Conference

Endemic fluorosis is usually found to occur with arsenicosis in many epidemic areas. Up until now, no patients with fluorosis have been found in the area of endemic iodine deficiency. The purpose of this investigation is to study the correlation between fluoride, arsenic, and iodine. Water samples were analysed for the concentration of fluoride, arsenic, and iodine as per the national standard method of China. 438 water samples were randomly selected from 5 counties. The detection rates of fluoride, arsenic, and iodine were 81.74% (358), 47.03% (206) and 98.63% (432), respectively. A linear relationship between fluoride and iodine was found in 2 counties

(Menyuan and Huanyuan, $P < 0.05$) and a linear relationship between fluoride and arsenic was found in Guide county ($P < 0.001$). The findings indicate that there is correlation between fluoride and arsenic; and between fluoride and iodine in the endemic areas of fluoride in China.

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Keywords: Arsenic; Correlation relations; Iodine; Regression.

EVALUATION OF CARIES INCIDENCE AND SEVERITY OF AGE-6 TEETH IN CHILDREN BETWEEN 7 AND 10 YEARS-OLD WITH DENTAL FLUOROSIS AND NON-FLUOROSIS

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P37

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Dental fluorosis is endemic, and recently its prevalence is also increasing related to the high use of fluor containing products in world. Dental fluorosis may occur in both primary and permanent teeth. Dental caries is an infectious disease. Carious lesions may easily transmit to other teeth. Inadequate oral hygiene behaviours and cariogenic nutrition may heighten the incidence and severity of dental caries. Age-6 teeth are the first foliated permanent teeth in oral cavity. They are firstly affected from caries because of their early-presence in oral environment, more consumption of cariogenic nutrients, and irregular oral hygiene behaviours in childhood. This study evaluated the caries incidence and severity of age-6 teeth in fluorosed and non-fluorosed children between 7–10 years-old in Isparta City which is an endemic fluorosis area in Turkey. In the study, the age-6 teeth of 532 children between 7–10 years-old with dental fluorosis (266) and non-fluorosis (266) and totalling 2128 were examined after getting the necessary permissions and consents for the children. The age-6 teeth of children were classified according to the Thylstrup-Fejerskov Index (TFI) as TFI=2–6 and TFI=0 (control-group) after the surfaces were mildly dried. The presence and intensity of caries were examined as “C(0)=no-caries, C(1)=caries in enamel, C(2)=advanced caries in dentin, C(3)=advanced caries in pulp, C(4)= caries on root surfaces”. Statistical analysis was performed with the Chi-Square Test in SPSS 15.0 computer programme ($p=0.0001$). The caries incidence was found to be significantly lower in the fluorosed children (14.7%) than in the non-fluorosed children (24.8%) ($p < 0.0001$). The caries severity was also found to be lower in fluorosed teeth statistically ($p < 0.0001$). Caries severity was mostly designated as C(1) in fluorosed and non-fluorosed teeth for all ages ($p < 0.0001$). Caries severity of C(2) was also common (15.6%) in all fluorosed and non-fluorosed teeth. The highest caries incidence was generally found in 9-years-old children (16.1%). Caries severity of C(1) was 9.4% and caries severity of C(2) was 6.2% in 9 years-old children. In both groups, the caries incidence was statistically higher in mandibula (29.5%) than maxilla (20%) ($p < 0.0001$). Caries incidence in mandibula was lower in fluorosed children than non-fluorosed children ($p < 0.0001$). In conclusion, the caries incidence and severity were found to be generally lower in fluorosed children than non-fluorosed children between 7 and 10 years-old.

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Keywords: Caries incidence in children; Caries severity in children; Dental fluorosis.

INFLUENCES OF DENTAL FLUOROSIS AND DEPROTEINISATION TREATMENT ON SHEAR BOND STRENGTHS OF COMPOSITE RESTORATIONS IN PERMANENT MOLAR TEETH

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Dental fluorosis often causes esthetic problems with teeth. Bonding of composite restorations to enamel largely depends on acid-etching but fluorosed enamel is more resistant to acid-etching because of the hypermineralized outer surface. In hypomineralized subsurface areas, enamel is more porous and the pores are occupied by water as well as enamel secretory proteins. The subsurface organic network decreases bonding of composites on teeth as hypermineralized surface. Some treatments were suggested with acid-etching to increase the bonding capacity of composites to the fluorosed teeth such as deproteinisation. Deproteinisation may be performed with an organic-solvent. Sodium hypochlorite (NaOCl) is known to be an excellent organic-solvent material that should be capable to remove the excess enamel protein. *In-vitro* shear bond strength (SBS) tests are commonly used to determine the bond strength of composites on teeth. This study aimed to evaluate the effects of dental fluorosis and deproteinisation treatment with NaOCl on SBS of composite restorations on permanent molar teeth. A total of 45 permanent molar teeth, freshly extracted for periodontal or orthodontic reasons, were separated using the Thylstrup-Fejerskov Index (TFI) as TFI=0 (control), TFI=1–3, and TFI=4–6 (15 teeth for each score) after disinfecting with distilled water containing 0.1% thymol (24h). Test procedures were determined ($n=10$ specimens for each procedure): Procedure-A= Acid-etching/60s; Procedure-AD=Acid-etching/60s+Deproteinisation/2min; Procedure-DA= Deproteinisation/2min+Acid-etching/60s. Totalling 90 cylinder-shaped composite specimens (Charisma, Heraeus-Kulzer/Germany) (3mm diameter \times 3mm height) were bonded with bonding agent (Gluma-Comfort-Bond, Heraeus-Kulzer/Germany) after test procedures applied with 35% phosphoric acid (Multipurpose-Etching-Gel, 3m/USA) and 5% NaOCl on mid-buccal or mid-palatinal/lingual enamel surfaces. Specimens were tested with universal-test machine (Lloyd-Lrx-Universal, Lloyd-Instruments/UK) (crosshead-speed; 1mm/min) after thermocycling (500 \times). Fracture types were determined under stereomicroscope (Leica, mz-12/ch94-35/Switzerland) (25 \times) as “adhesive, mixed, cohesive”. Data were determined with two-way-anova and Sheffe’s multiple range tests ($p < 0.05$). In TFI=0 and TFI=1–3 groups; statistical differences were not between three procedures ($p > 0.05$). In TFI=4–6 groups; Procedure-AD has showed the highest SBS values. Values of Procedure-A (mean: 24.02 \pm 1.88 MPa) were statistically lower than values of Procedure-AD (mean: 30.51 \pm 1.19 MPa) ($p < 0.05$). Fracture types were mostly found “adhesive” in TFI=0 (56.66%), “mixed” in TFI=1–3 (46.66%), and “cohesive” in TFI=4–6 (60%). Conclusion: Dental fluorosis and deproteinisation treatment affected the SBS of composite restorations on

permanent molar teeth.

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Keywords: Acid-etching; Dental fluorosis; Deproteinisation; Shear bond strength.

ESTIMATION OF THE NUMERICAL DENSITY OF ODONTOBLAST CELLS IN FLUOROTIC AND NON-FLUOROTIC TOOTH PULP

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Fluoride has been used for decades due to its caries preventive properties, either systemically or topically, in spite of its link with dental fluorosis. It is essential to understand all the potential clinical effects of fluoride on the pulpal cells, especially the odontoblasts. Odontoblasts are responsible for the continual secretion and repair of dentine.

In the event of dentine injury, the capacity to secrete reactionary dentine is depend on the vitality of the odontoblast layer. The aim of this study was to compare the numerical density of odontoblast cells in fluorotic and non-fluorotic tooth pulp. A total of 28 caries free and freshly extracted primary first molar teeth obtained from 9–11 year old children with and without fluorosis were used. Root resorption levels were not more than 1/3 of the root length in all study groups. The numerical density of odontoblast cells in the coronal pulps were estimated using unbiased stereological principles and the optical disector method. Extracted teeth were prepared according to routine histological techniques for light microscopic investigation. Histological sections were arranged in order to randomize systematic sampling techniques and then the numerical densities of the odontoblast cells were determined using a computer assisted stereological research system. The numerical densities of the odontoblast cells in the maxillary and mandibular first primary molar teeth in the fluorosis group were found to be lower than in the healthy group, however the difference between the groups was not statistically significant ($p>0.05$). Additionally, there was no statistically significant difference between gender and the numerical densities in patients with and without fluorosis ($p>0.05$). These findings indicate that the number of odontoblast cells are not influenced extensively from the fluoride content of fluorotic tooth pulp and it seems that there is need for further investigations on odontoblast cells of teeth with fluorosis.

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Keywords: Dental fluorosis; Odontoblasts; Numerical density; Stereology.

SALIVARY SIALIC ACID LEVELS OF THE CHILDREN LIVING IN AN ENDEMIC FLUOROSIS AREA

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It is generally accepted that saliva is of paramount importance for maintenance of oral health. Saliva contains many components. Proteins are constitutes the organic structure of the saliva and the most of the salivary proteins are glycoproteins. Sialic acid is an important structural component of glycoprotein and playing a part in enhancing bacterial agglutination. The beneficial effect of fluoride in the prevention of dental caries has been

known for many years in spite of its link with dental fluorosis and Isparta is one of the endemic fluorosis areas in Turkey. There have been no studies on salivary sialic acid levels in children living in an endemic fluorosis area. The purpose of this study was to determine the sialic acid levels and to examine streptococcus/lactobacillus amount of the saliva, collected from children with and without dental fluorosis, living in an endemic fluorosis area. Thirty-six healthy 8–10 year-old-children were included in the study after receiving informed consent from their parents. They were assigned to three groups; (Group A, $n=12$) children with dental fluorosis and living in an endemic fluorosis area, (Group B, $n=12$) children without dental fluorosis and living in an endemic fluorosis area for 2 years, (Group C, $n=12$) children with healthy teeth and living in low fluoridated area. Unstimulated and paraffin-chewing stimulated saliva were collected from the children to determine the sialic acid levels and to examine the streptococcus and lactobacillus amount. The sialic acid levels were significantly lower in children living in an endemic fluorosis area without the importance of dental fluorosis ($p=0.009$) Among the groups; there was a statistically significant relationship between Group A/C ($p=0.006$) and Group B/C ($p=0.008$). Furthermore, no statistically significant difference was observed for gender and for amount of streptococcus and lactobacillus in any groups. The findings of our study show that sialic acid levels, derived from the saliva, which was collected from the children, who had been living in an endemic fluorosis area, were significantly lower.

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Keywords: Fluorosis, endemic; Saliva; Sialic acid.

PRESENT SITUATION OF ENDEMIC FLUOROSIS AFTER IMPROVING COOKING STOVES IN THE RURAL AREA OF ZHEJIANG PROVINCE

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In order to know the situation of endemic fluoride poisoning after the improved cooking stove is introduced as intervention strategy, we made a follow-up assessment in an endemic area of Fancangun village, Zhejiang province, China. Prior to the start of the project, individuals were randomly selected and examined for dental fluorosis. The fluoride content in samples of water, indoor air, coal, soil, and rice were analysed. The fluoride content of

household drinking water was found to be 0.715 ± 0.697 mg/L, which is higher than the fluoride level in the raw water from the well, 0.483 ± 0.206 mg/L ($t=3.16$, $P<0.01$). The indoor air fluoride concentration in the households was about 0.02 mg/m³, while the fluoride level in the indoor air of households using coal for fuel

was 0.052 ± 0.037 mg/m³, which is higher than that of households using other fuel (0.014 ± 0.008 mg/m³, $P < 0.01$). The fluoride content of the urine of children living in families using coal as fuel was 0.638 ± 0.425 mg/L which was higher than that of those living in families using other fuel (0.304 ± 0.285 mg/L, $t = 2.895$, $P < 0.01$). The fluoride concentration in the soil was 2.998 ± 2.165 mg/kg with the highest level being 5.966 mg/kg. The fluoride content in the rice was 0.858 ± 0.196 mg/kg, which was lower than the Government's standard (1.0mg/kg). A fluoride concentration higher than 1.0mg/kg was present in 22.22% (2/9) of the rice samples. The incidence of dental fluorosis in children aged 8-12 years old was 7.43% (26/350). Compared with the findings of 1990, there was an obvious decrease in the incidence of dental fluorosis in the children. Fluorosis prevention and monitoring should be continuously carried out further in the future.

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Keywords: Coal fuel pollution; Endemic fluoride poisoning; Epidemiology of dental fluorosis.

THE USE OF A PORTABLE X-RAY PROTECTIVE BELLOWS SHIELD IN THE RADIOLOGICAL INVESTIGATION OF ENDEMIC DISEASES

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The purpose of this study is to develop a portable X-ray protective bellows shield to give improved protection when taking radiographs as part of the investigation of endemic diseases. In the routine method of taking radiographs, we fixed the X-ray bulb tube on a plank and radiographed the forearm and (lower) leg with the doctor standing 1-2 m from X-ray bulb tube center. A pressurized ionization survey instrument was used to survey the radiation exposure for the subjects, in the forehead, chest, and abdomen, both with and without the protective shield. The quality of the radiographs and the X-ray exposure for 55 subjects were studied. For radiographing the forearm, the X-ray dosages for the conventional method were 953, 825 and 856 respectively while, with the protective shield, the corresponding rates were 1.7, 2.9 and 7.9 respectively. For radiographing the (lower) leg, the corresponding rates for the two groups were 1200, 1302, 1085 and 2.2, 3.4, 9.1. The X-ray dosage exposure for the protective shield group was smaller (1/100-1/500) than that for the group given the routine protection and using the protective shield produced better radiographs, with a 30% higher top grade rate than that obtained with the regular method. The portable X-ray protective bellows shield was functional and reliable, gave better protection, and had a higher efficiency for the radiological investigation of endemic diseases.

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Keywords: Endemic disease investigation; Protective bellows shield; Radiography.

THE SOURCE OF THE FLUORIDE IN THE GROUNDWATER IN THE GANZHOU DISTRICT OF GANSU PROVINCE

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In order to solve the problem of drinking water safety in the drinking-water endemic fluorosis area in the Ganzhou District of Gansu Province, we carried out a hydrogeological survey with tests on rock, soil, and water samples. We found that the source of the fluoride in the groundwater was from fluorine weathered rock, which extended from Qilian mountain to Longshou mountain. The fluoride content in the groundwater of the quaternary unconsolidated layers in the front of Longshou mountain was higher than that of Qilian mountain. The fluoride levels in the groundwater increased from the piedmont gravelly plain to the fine earth plain and showed horizontal zonation. The fluoride level was found to reduce with increased depth in the fine earth plain and displayed vertical zonation. We discussed the evolution and enrichment of the fluoride content in the groundwater in terms of the depositional environment, geochemical action, and hydrodynamic field. We confirmed that the safe aquifers for supplying drinking water are in the depth range of 80-100 m.

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